

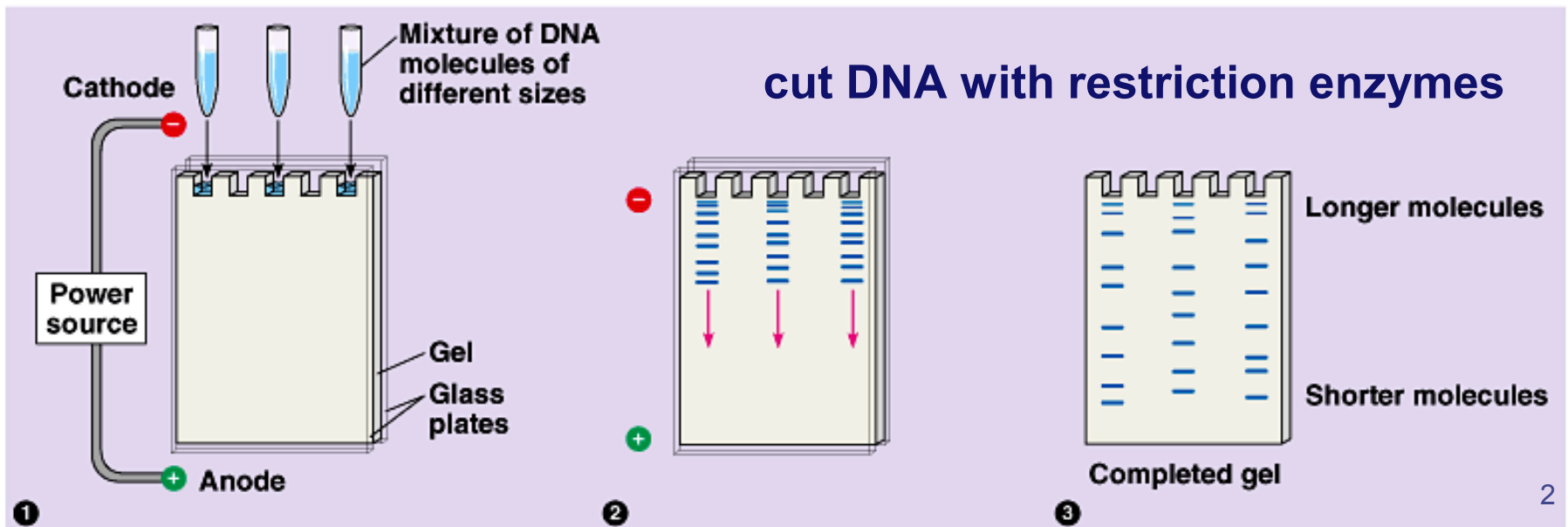


Advanced Techniques

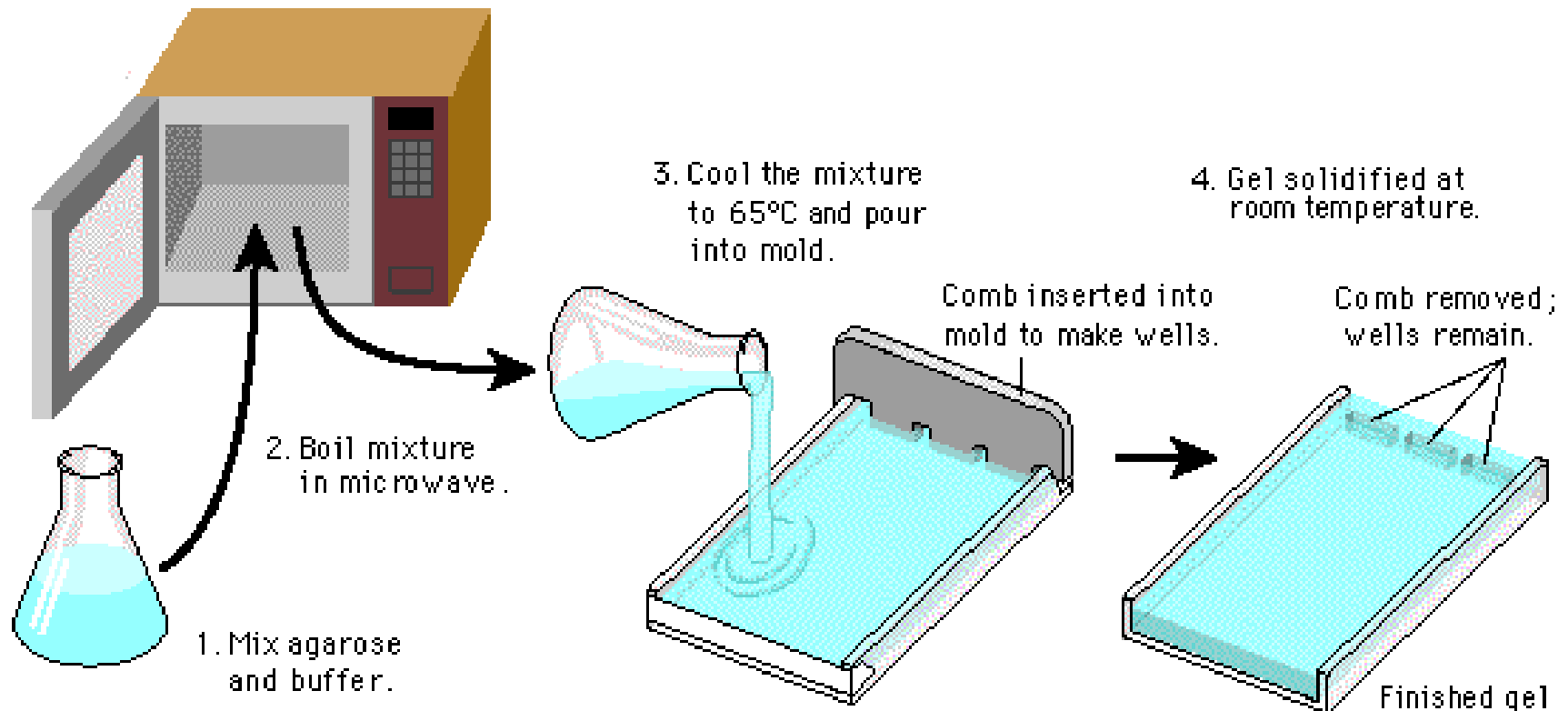
Electrophoresis & RFLPs

Gel Electrophoresis

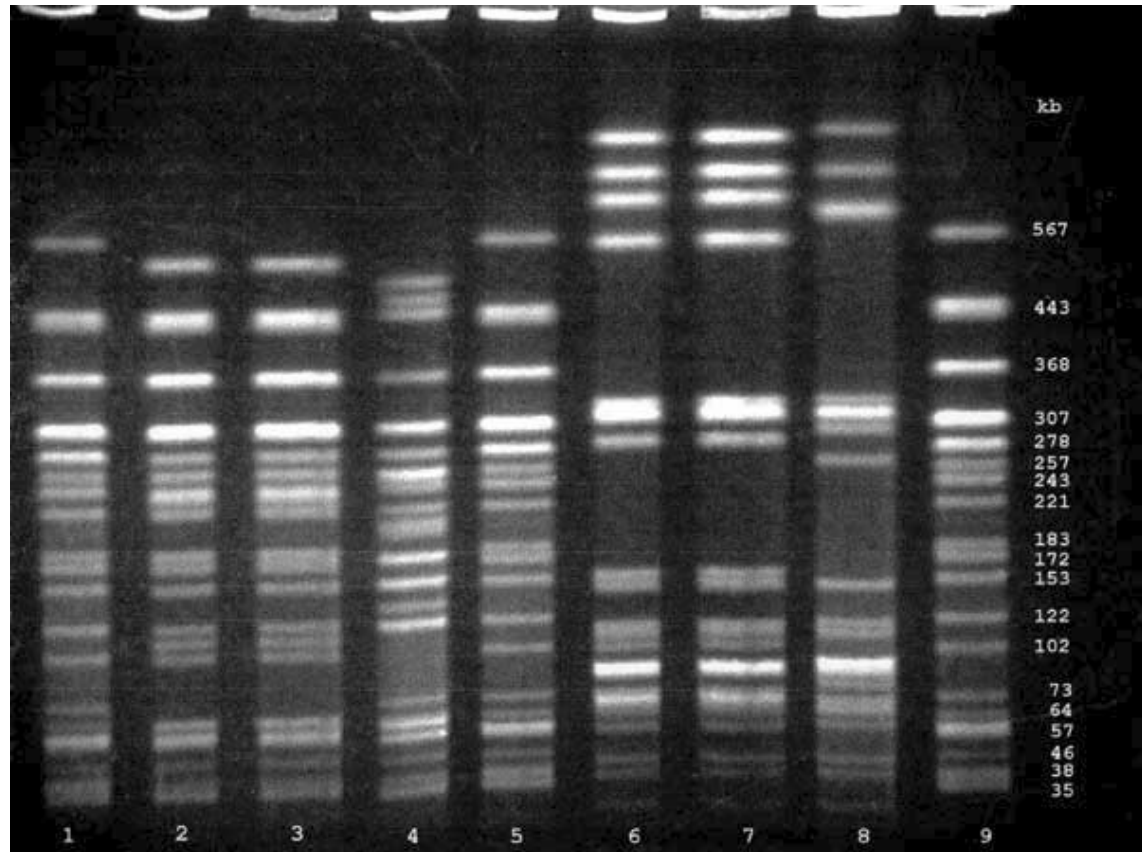
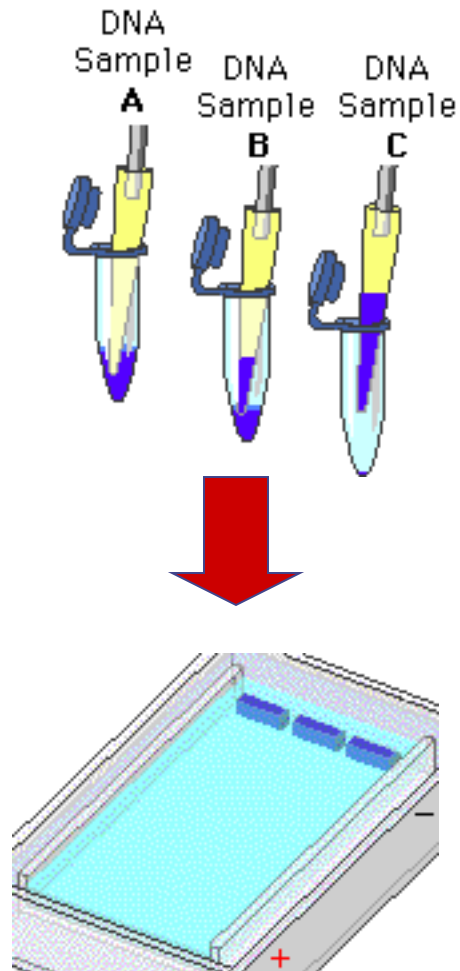
- Separation of DNA fragments by size
 - ◆ DNA is negatively charged
 - moves toward + charge in electrical field
 - ◆ agarose gel
 - “swimming through Jello”
 - smaller fragments move faster



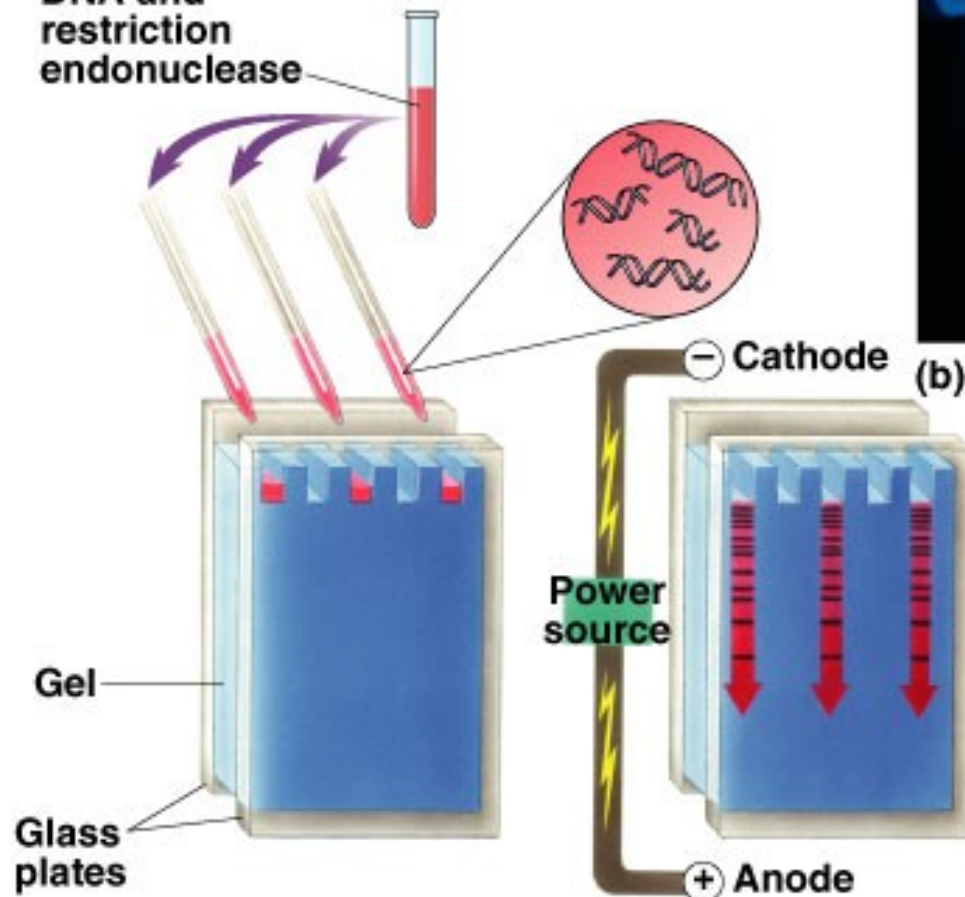
Gel Electrophoresis



Gel Electrophoresis



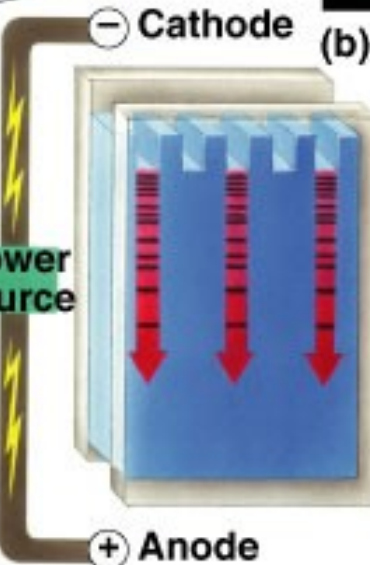
DNA and restriction endonuclease



Mixture of DNA fragments of different sizes in solution placed at the top of "lanes" in the gel



(b)



Electric current applied, fragments migrate down the gel by size—smaller ones move faster (and therefore go farther) than larger ones

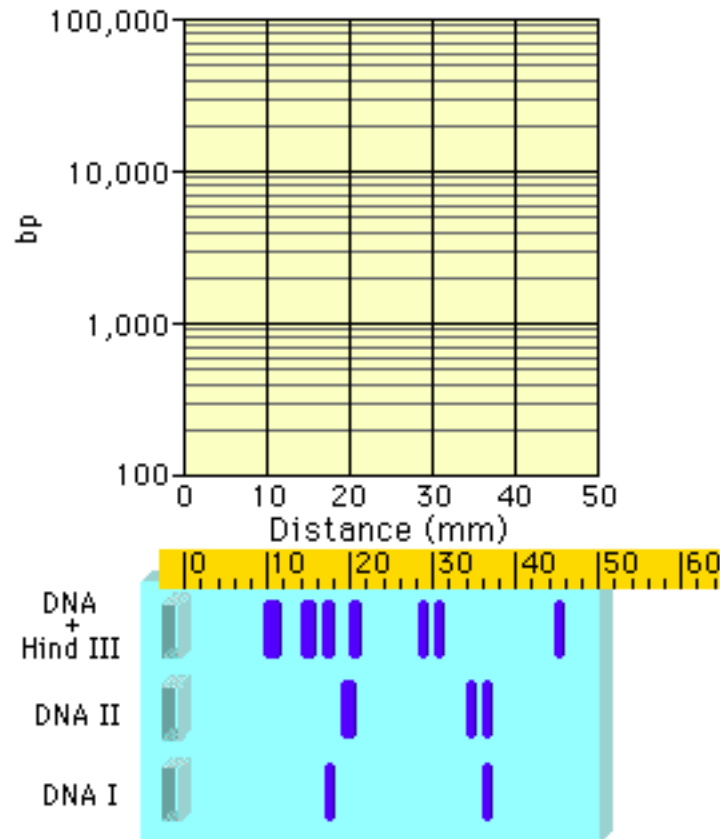
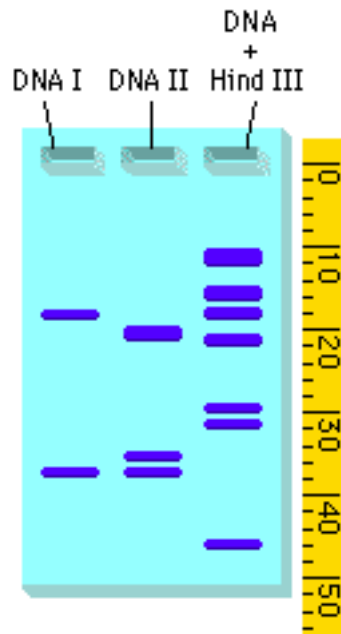


Longer fragments

Shorter fragments

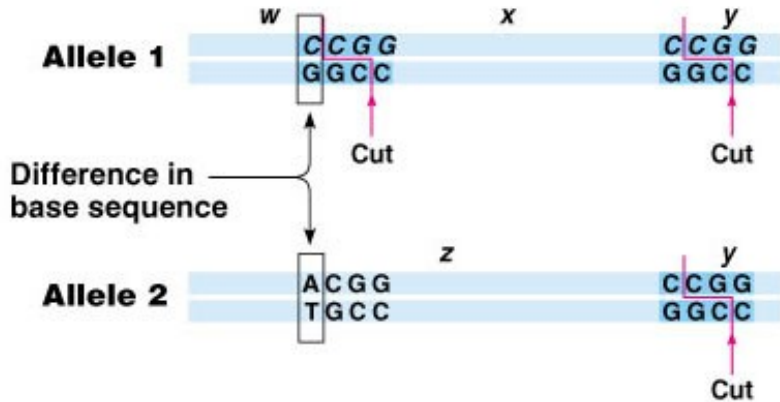
Measuring fragment size

- compare bands to a known “standard”
 - ◆ usually lambda phage virus cut with HindIII
 - nice range of sizes with a distinct pattern

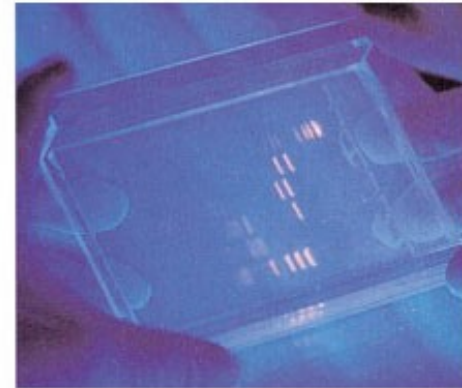


RFLP

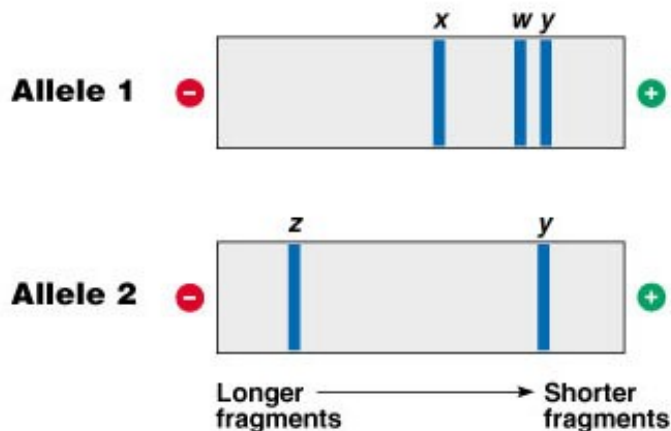
- **Restriction Fragment Length Polymorphism**
 - ◆ differences in DNA between individuals



(a) DNA from two alleles



(c) Completed gel

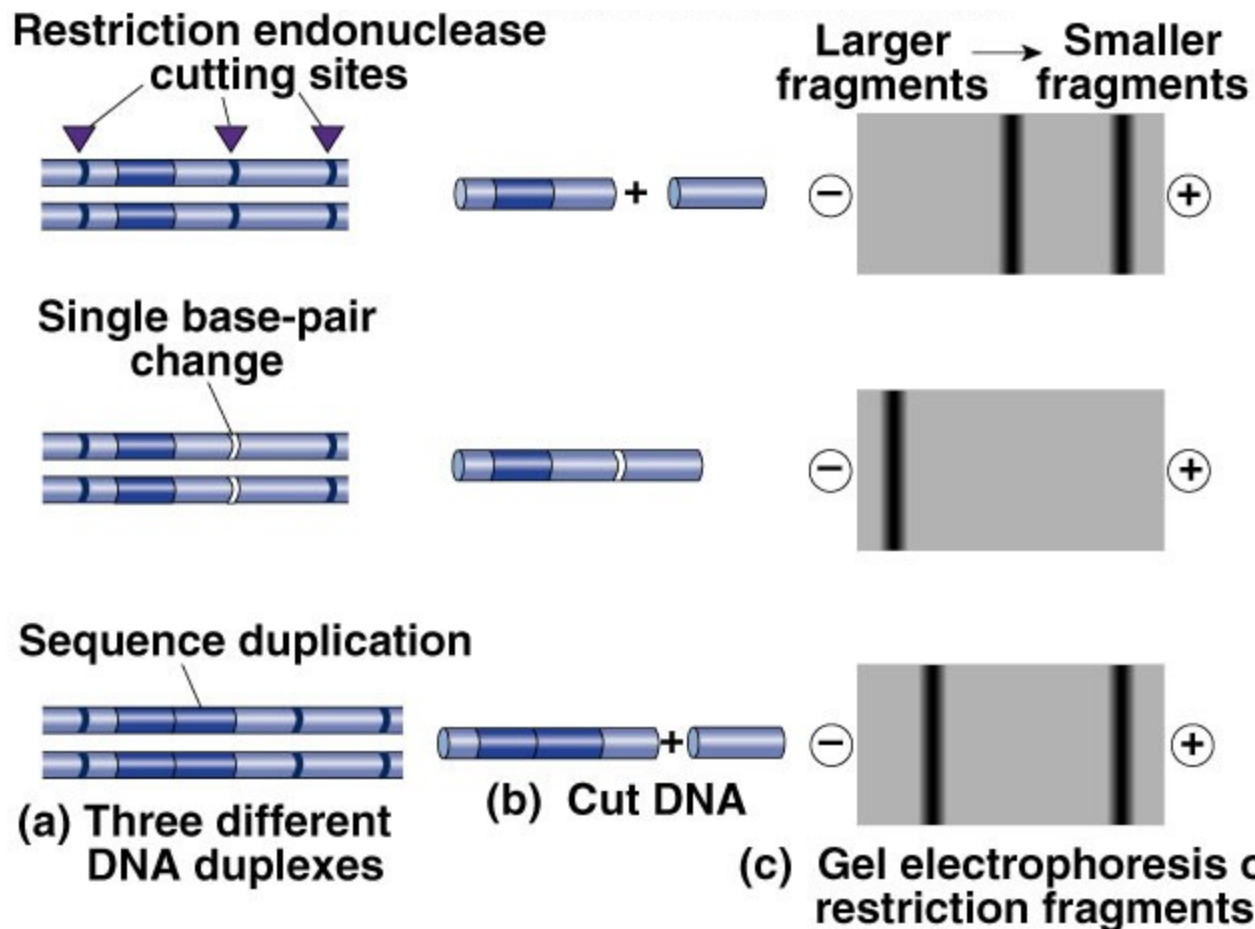


(b) Electrophoresis of restriction fragments

- ◆ change in DNA sequence affects restriction enzyme “cut” site
- ◆ will create different band pattern

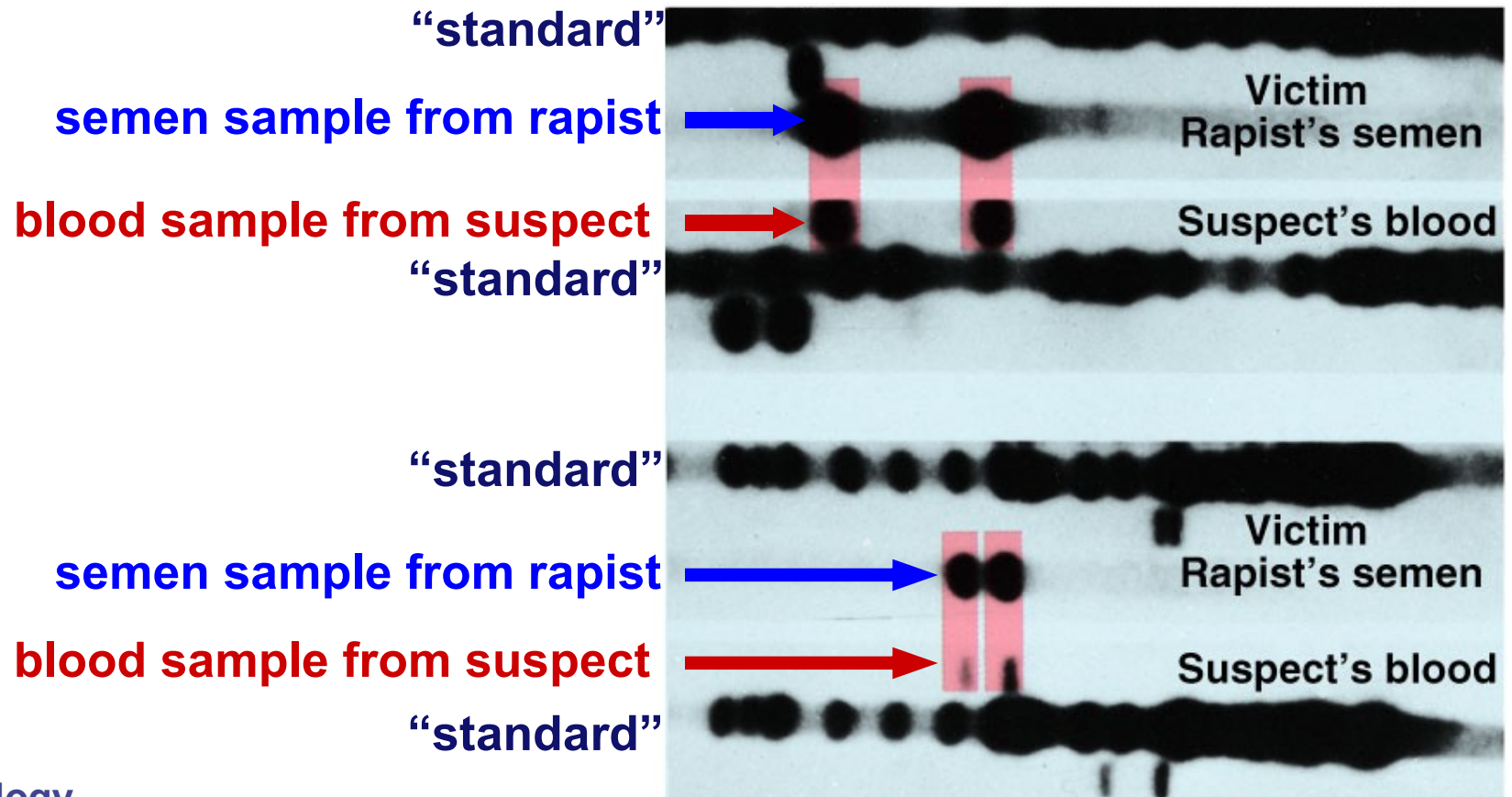
Polymorphisms in populations

- Differences between individuals at the DNA level



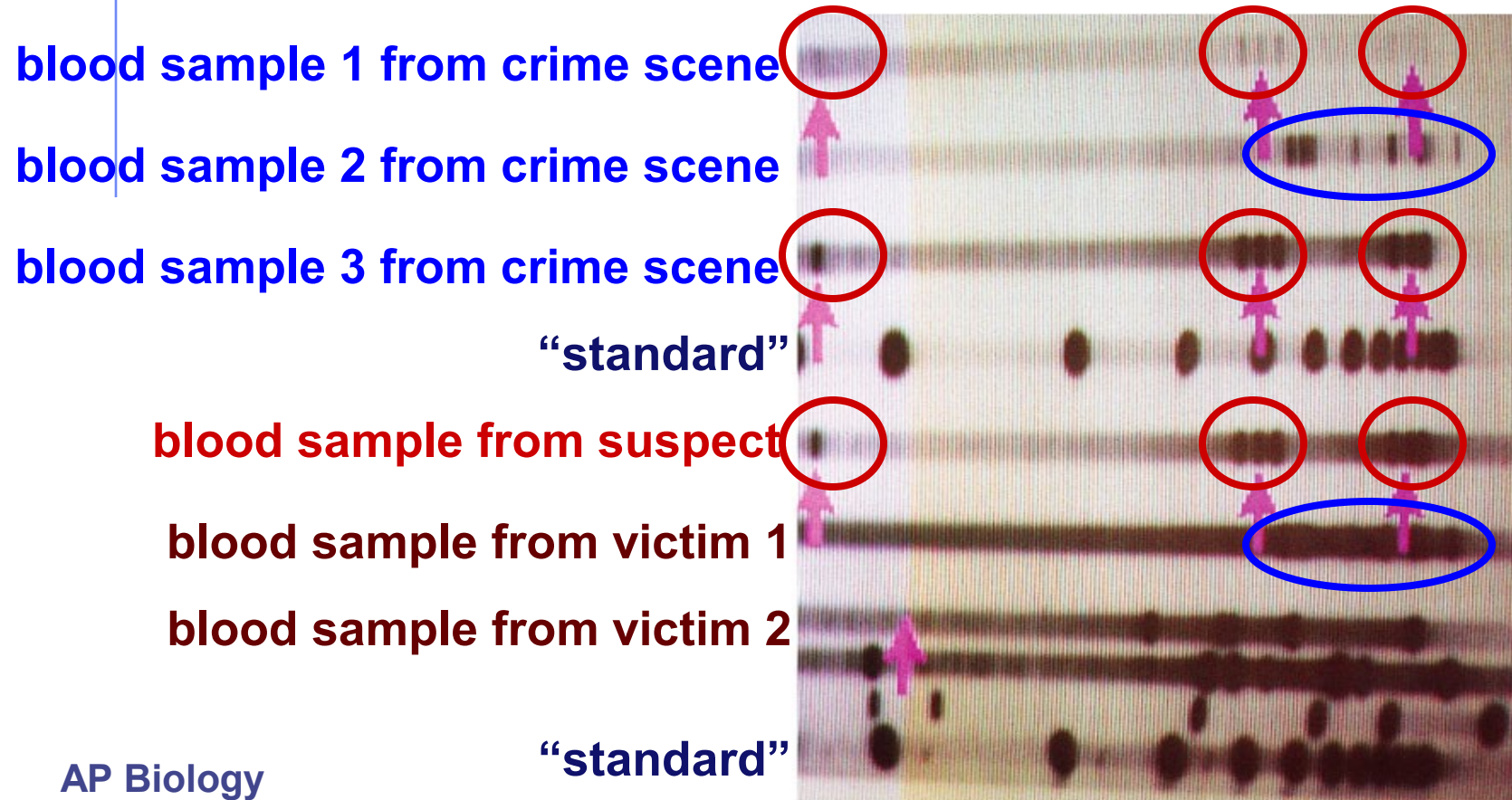
RFLP use in forensics

- 1st case successfully using DNA evidence
 - ◆ 1987 rape case convicting Tommie Lee Andrews



RFLP use in forensics

- Evidence from murder trial
 - ◆ Do you think suspect is guilty?





Any Questions??

RFLP use in forensics

- Evidence from murder trial
 - ◆ Do you think suspect is guilty?

blood sample 1 from crime scene

blood sample 2 from crime scene

blood sample 3 from crime scene

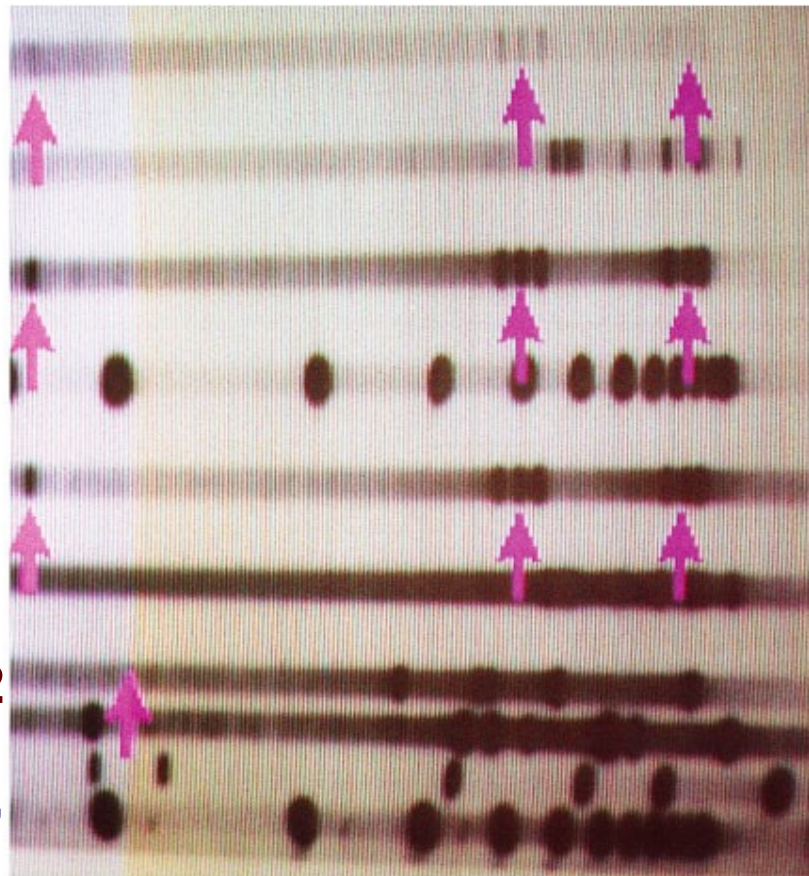
“standard”

blood sample from suspect

blood sample from victim 1

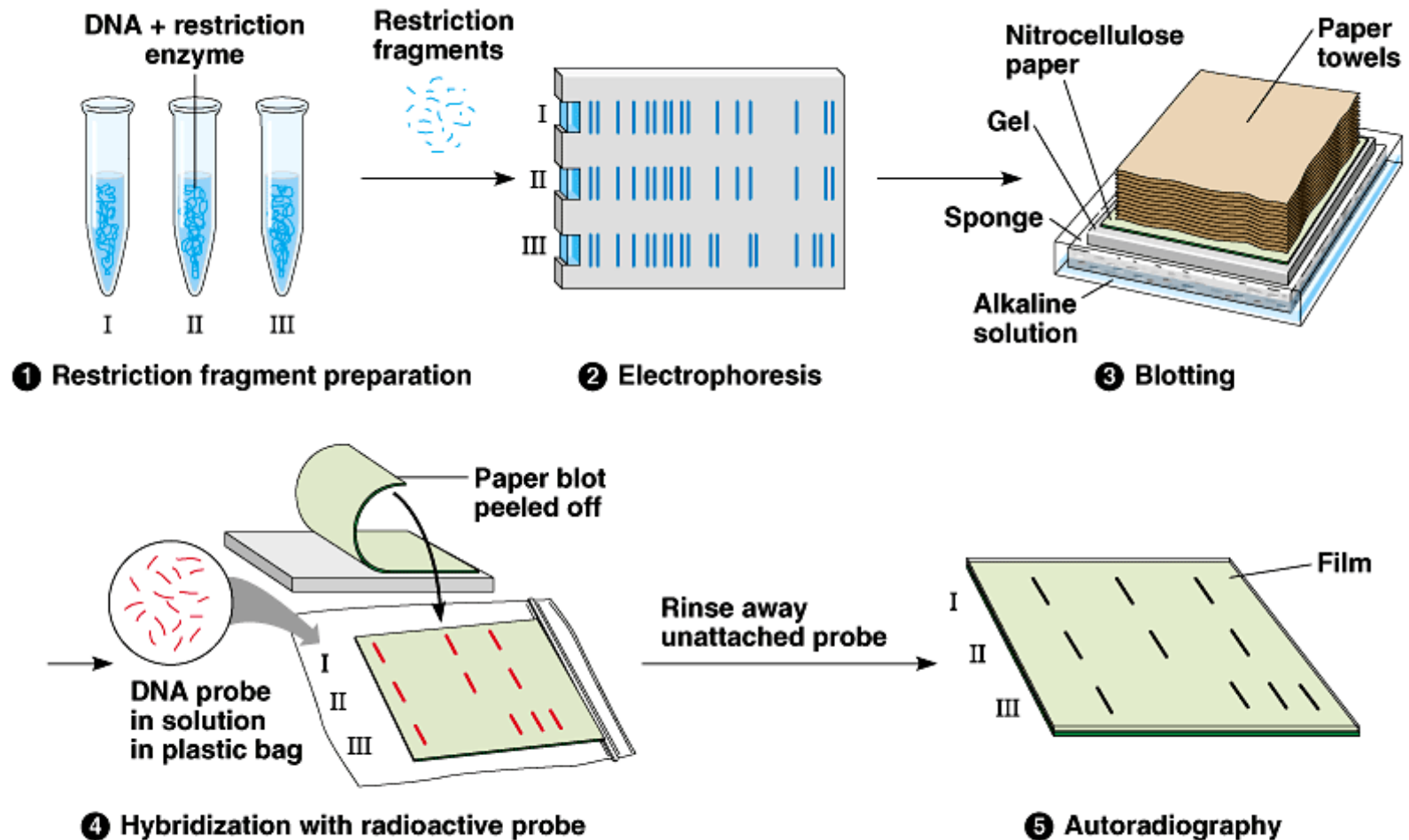
blood sample from victim 2

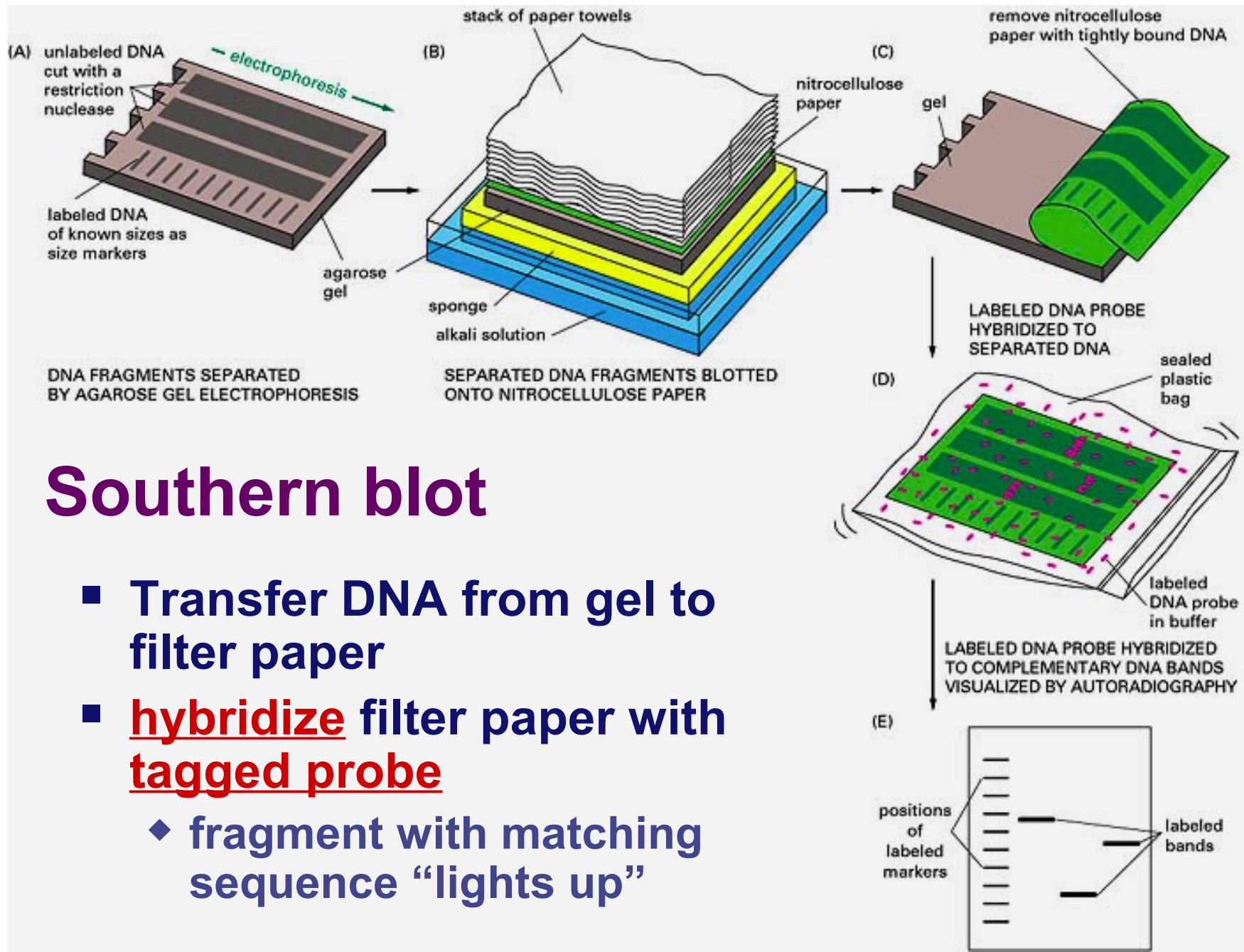
“standard”



Southern Blot

- Want to locate a sequence on a gel?



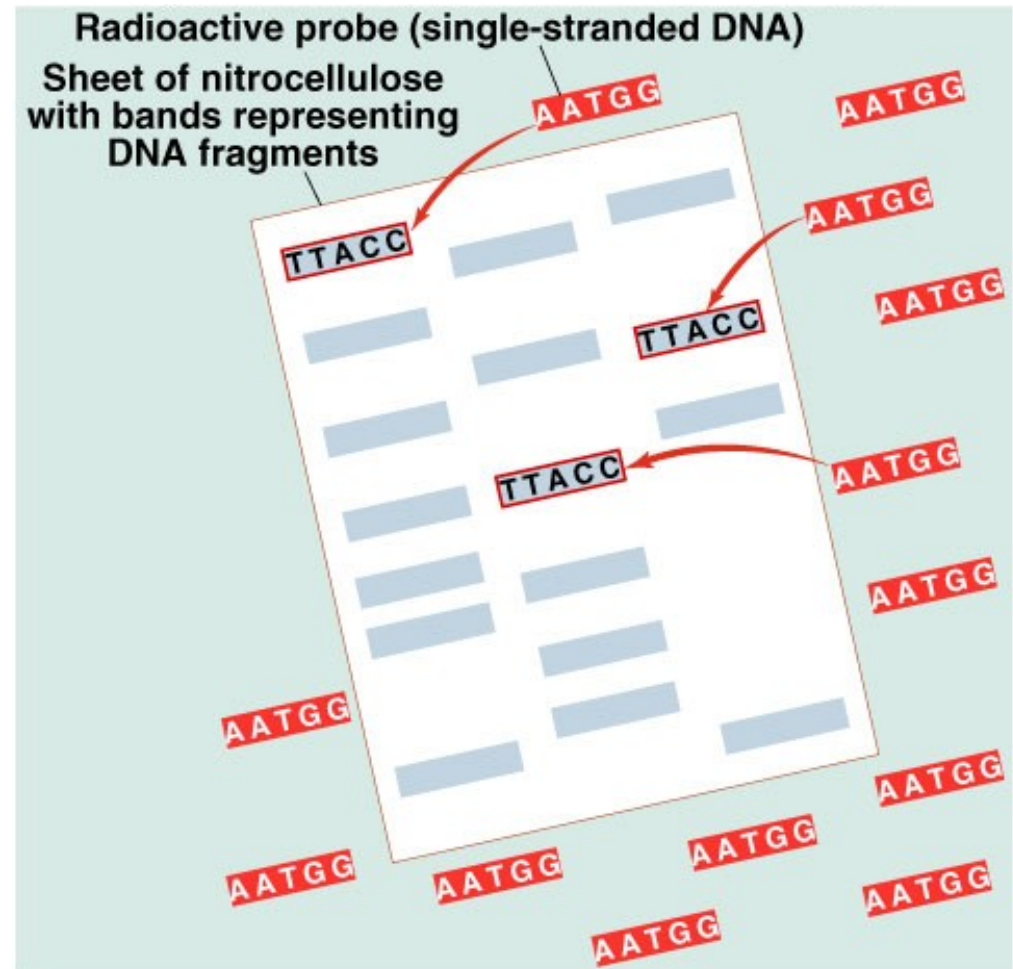


Southern blot

- Transfer DNA from gel to filter paper
- hybridize filter paper with tagged probe
 - ◆ fragment with matching sequence “lights up”

Hybridization in Southern Blotting

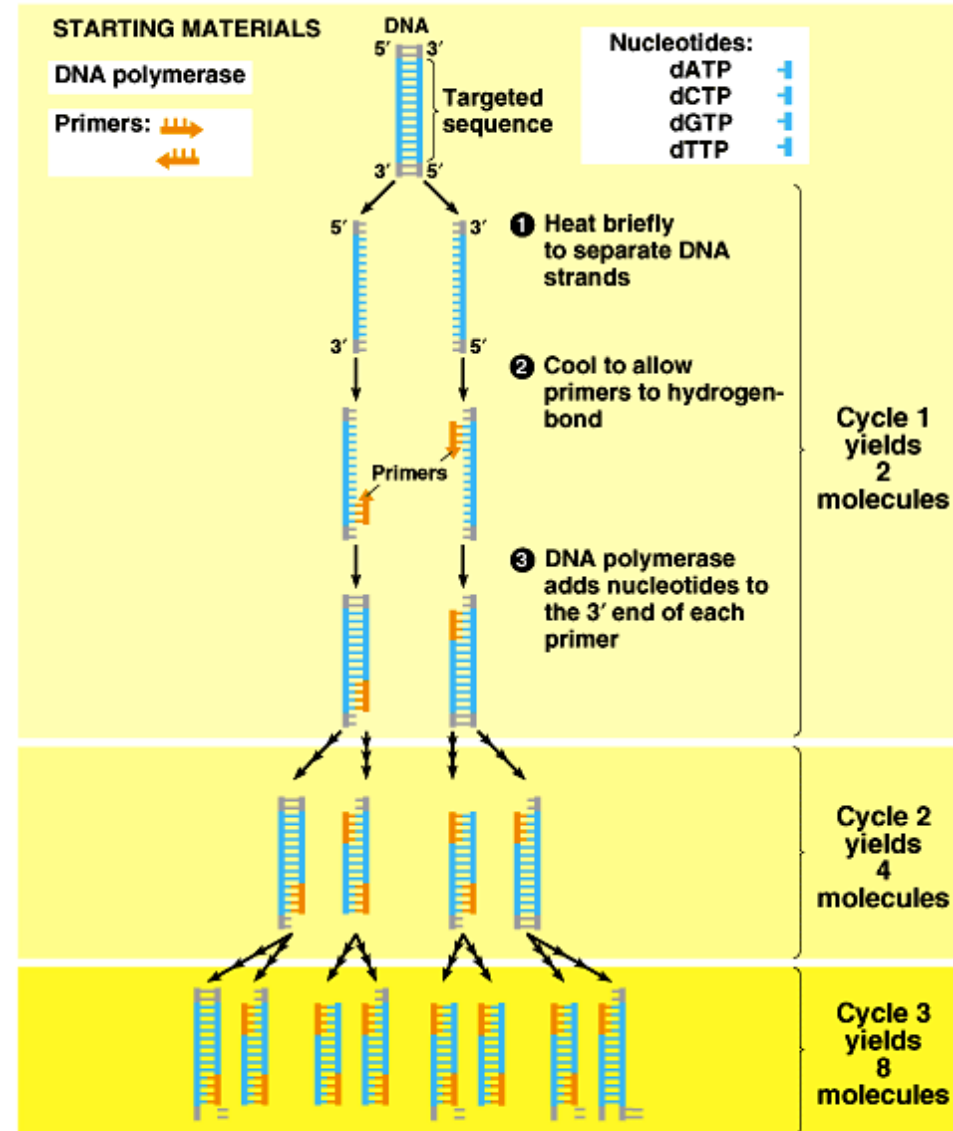
- Use radioactive probe to locate gene on filter paper
 - ◆ go back to gel & cut out piece of DNA you want to collect



Polymerase Chain Reaction (PCR)

- What if you have too little DNA to work with?
 - ◆ PCR is a method for making many copies of a specific segment of DNA
 - ◆ ~only need 1 cell of DNA to start

copying DNA without bacteria or plasmids!



PCR process

- It's copying DNA in a test tube!
- What do you need?
 - ◆ template strand
 - ◆ DNA polymerase enzyme
 - ◆ nucleotides
 - ◆ primer



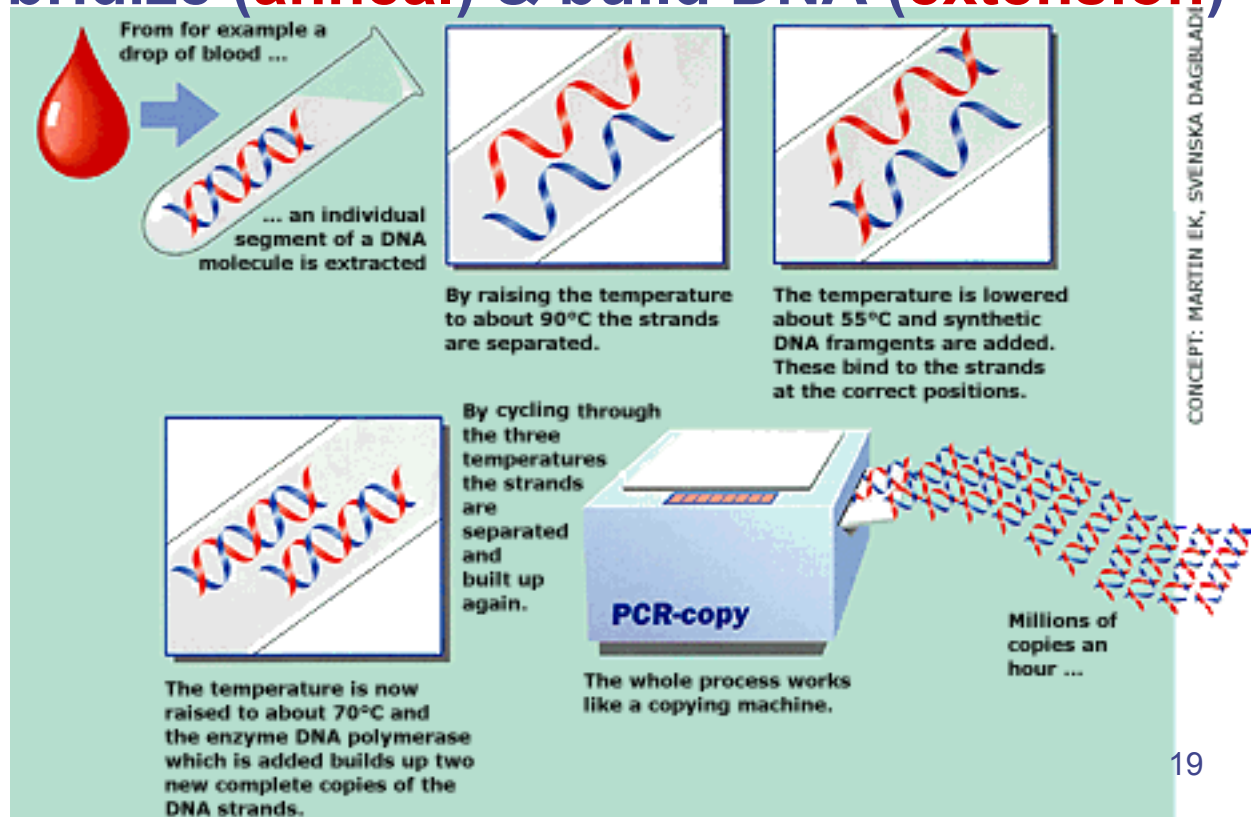
Thermocycler



PCR process

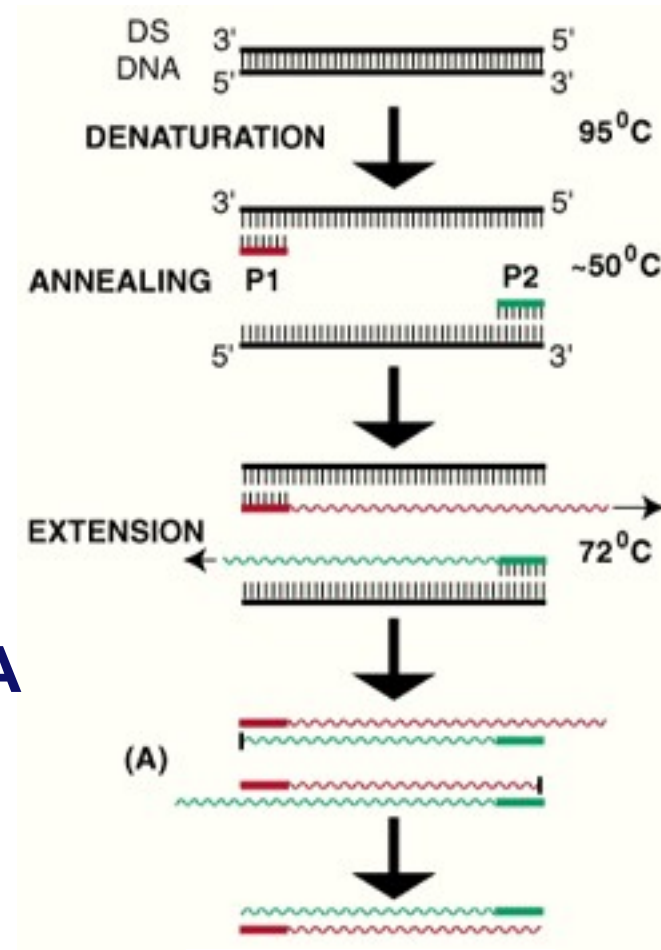
- What do you need to do?
 - in tube: DNA, enzyme, primer, nucleotides
 - heat (90°C) DNA to separate strands (**denature**)
 - cool to hybridize (**anneal**) & build DNA (**extension**)

What does 90°C do to our DNA polymerase?



PCR primers

- **The primers are critical!**
 - ◆ need to know a bit of sequence to make proper primers
 - ◆ primers bracket target sequence
 - start with long piece of DNA & copy a specified shorter segment
 - primers define section of DNA to be cloned



20-30 cycles
3 steps/cycle
30 sec/step

The polymerase problem

PCR

20-30 cycles
3 steps/cycle
30 sec/step

- Heat DNA to denature it
 - ◆ 90°C destroys DNA polymerase
 - ◆ have to add new enzyme every cycle
 - almost impractical!
- Need enzyme that can withstand 90°C...
 - ◆ Taq polymerase
 - from hot springs bacteria
 - ◆ *Thermus aquaticus*

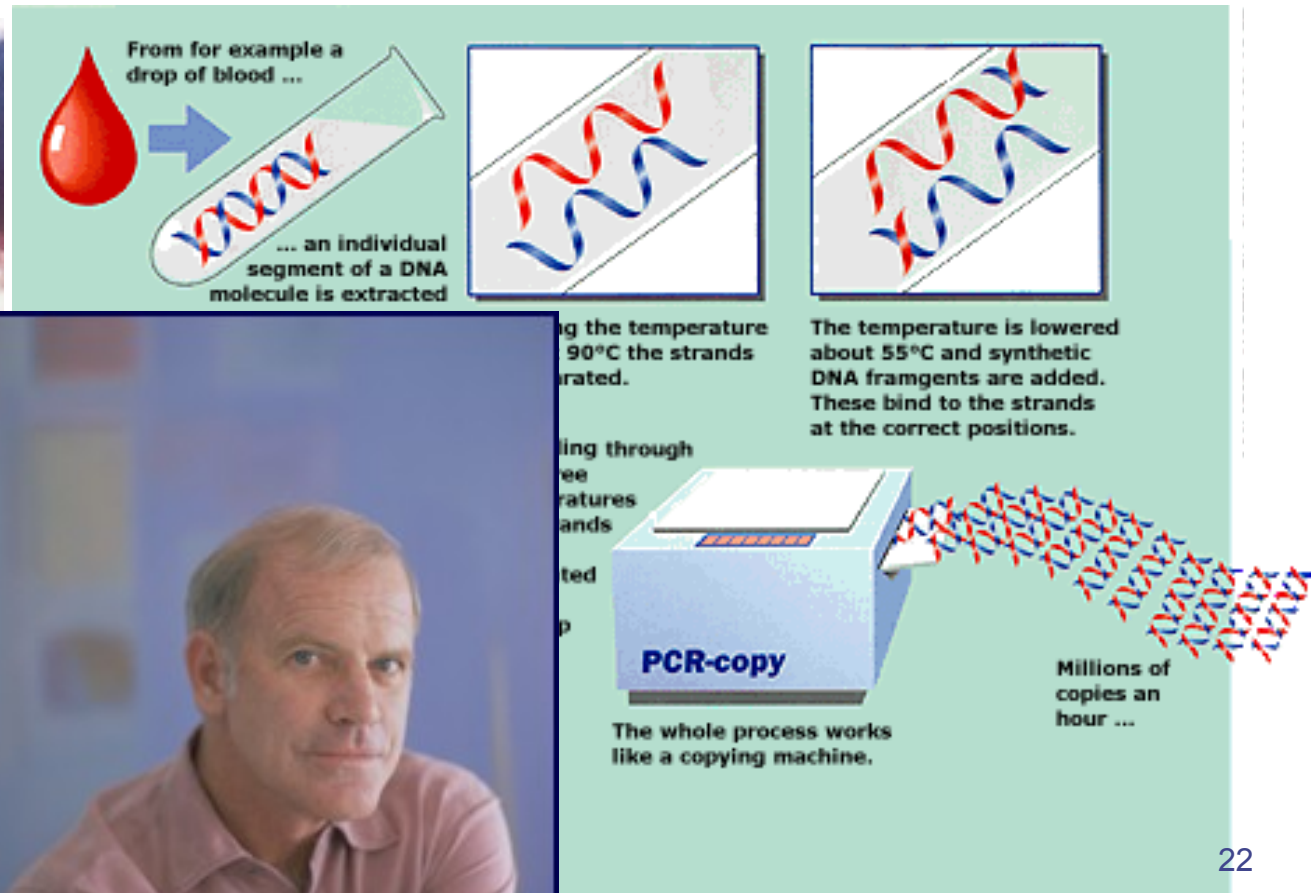
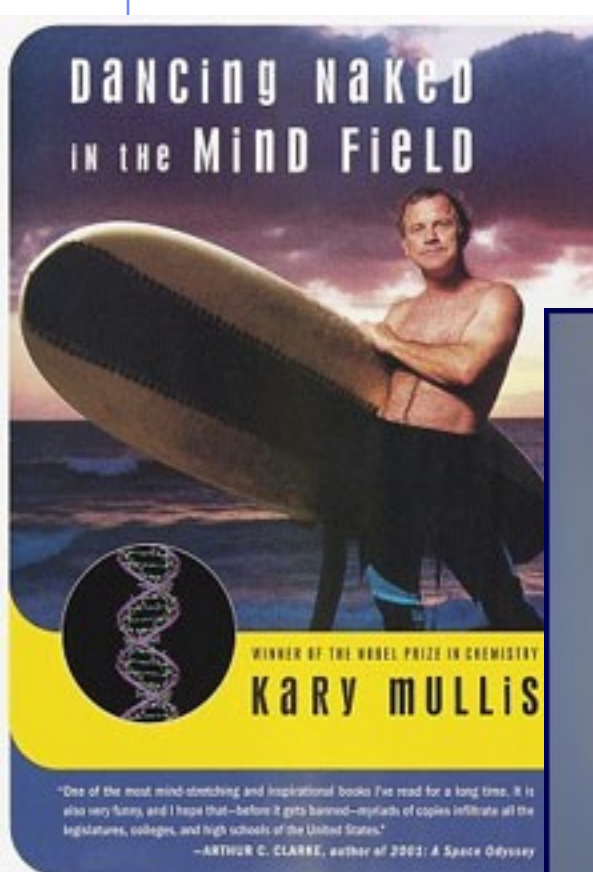


play DNAi movie

1985 | 1993

Kary Mullis

- development of PCR technique
 - ◆ a copying machine for DNA



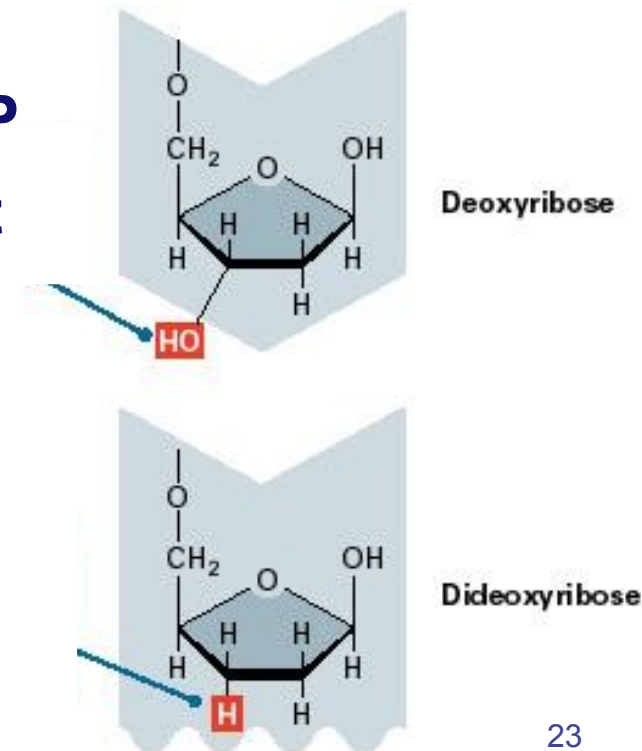
DNA Sequencing

■ Sanger method

- ◆ determine the base sequence of DNA

- ◆ **dideoxynucleotides**

- ddATP, ddGTP, ddTTP, ddCTP
- missing O for bonding of next nucleotide
- terminates chain



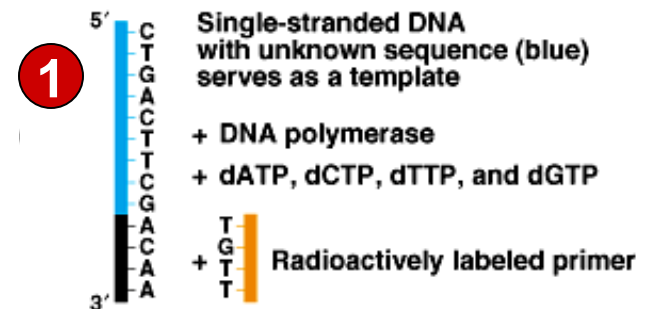
DNA Sequencing

■ Sanger method

- ◆ synthesize complementary DNA strand *in vitro*

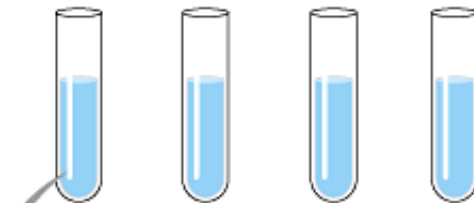
- ◆ in each tube:

- “normal” N-bases
- dideoxy N-bases
 - ◆ ddA, ddC, ddG, ddT
- DNA polymerase
- primer
- buffers & salt



Prepare four reaction mixtures

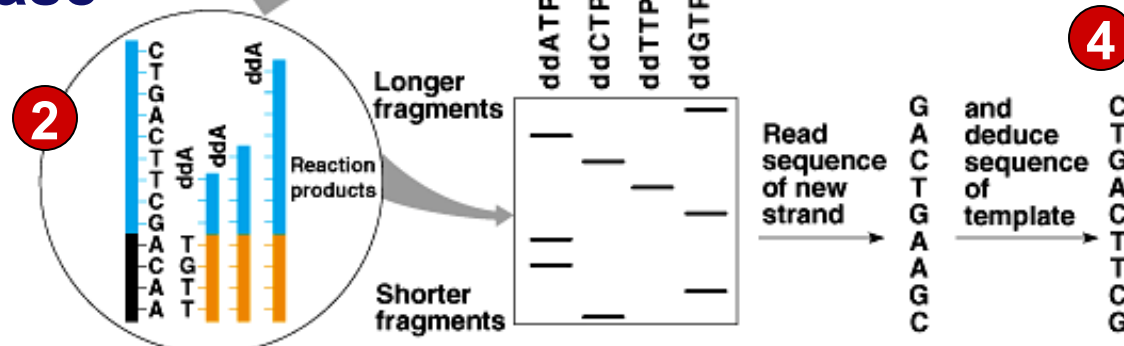
+ ddATP + ddCTP + ddTTP + ddGTP



DNA synthesis

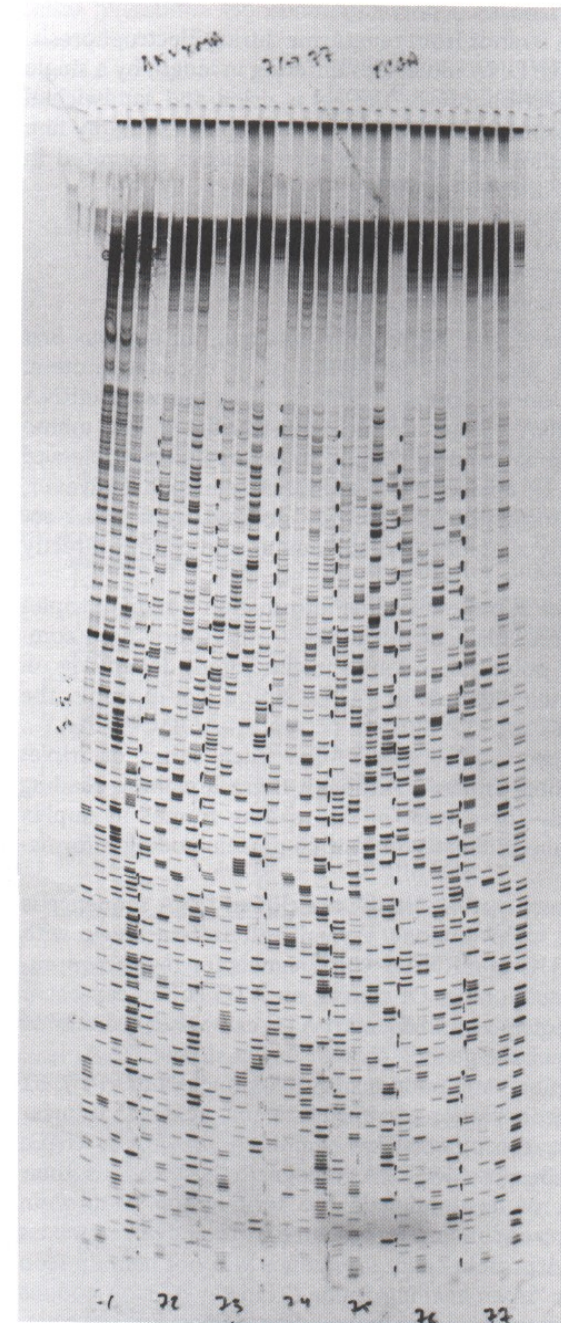
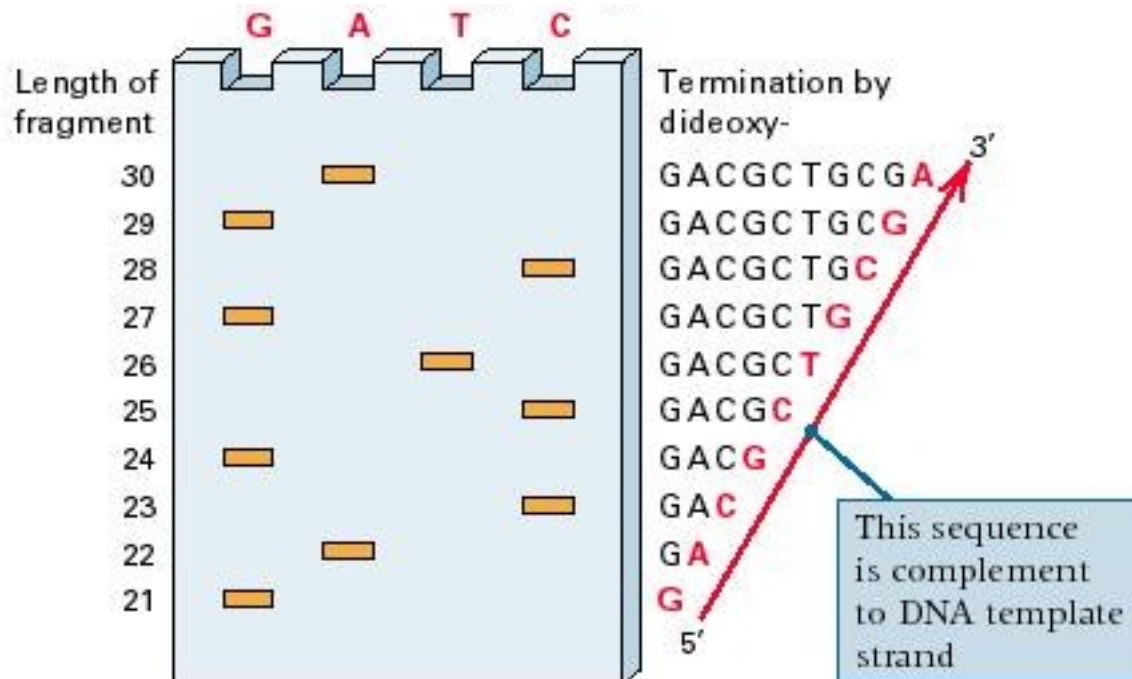
3

Gel electrophoresis followed by autoradiography



Reading the sequence

- Load gel with sequences from ddA, ddT, ddC, ddG in separate lanes
 - ◆ read lanes manually & carefully
 - ◆ polyacrylamide gel

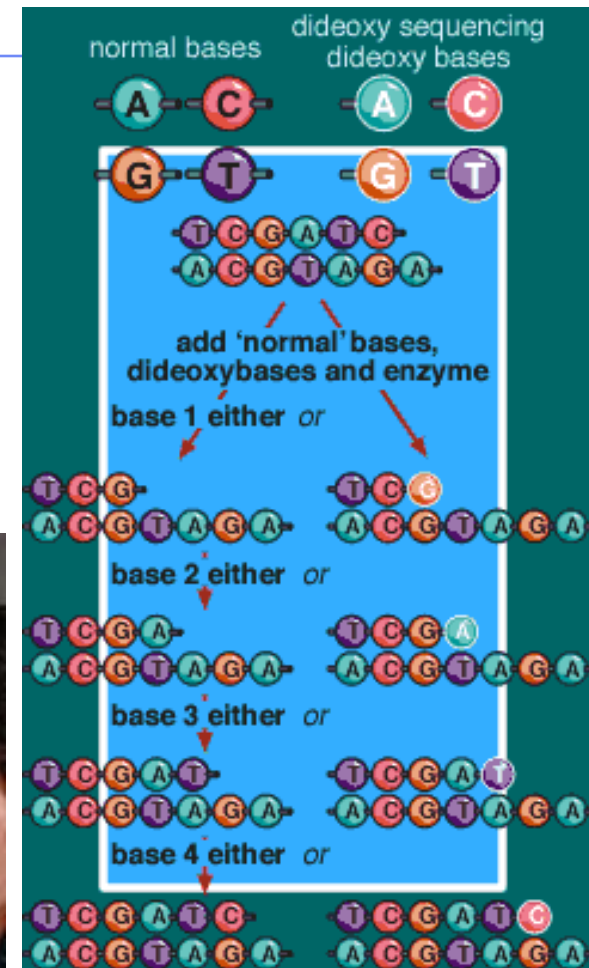
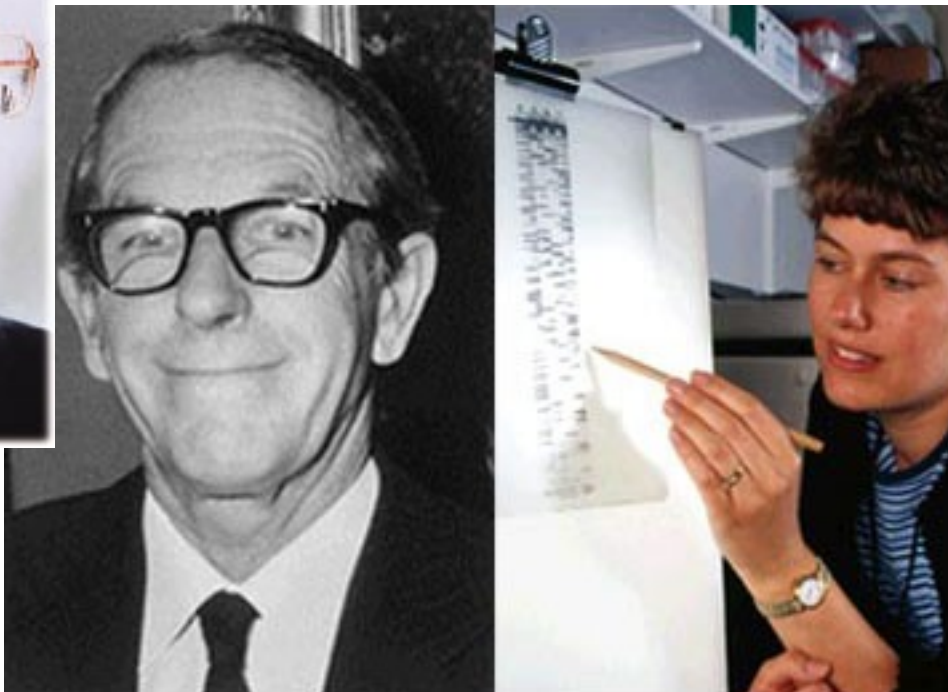


1978 | 1980

Fred Sanger

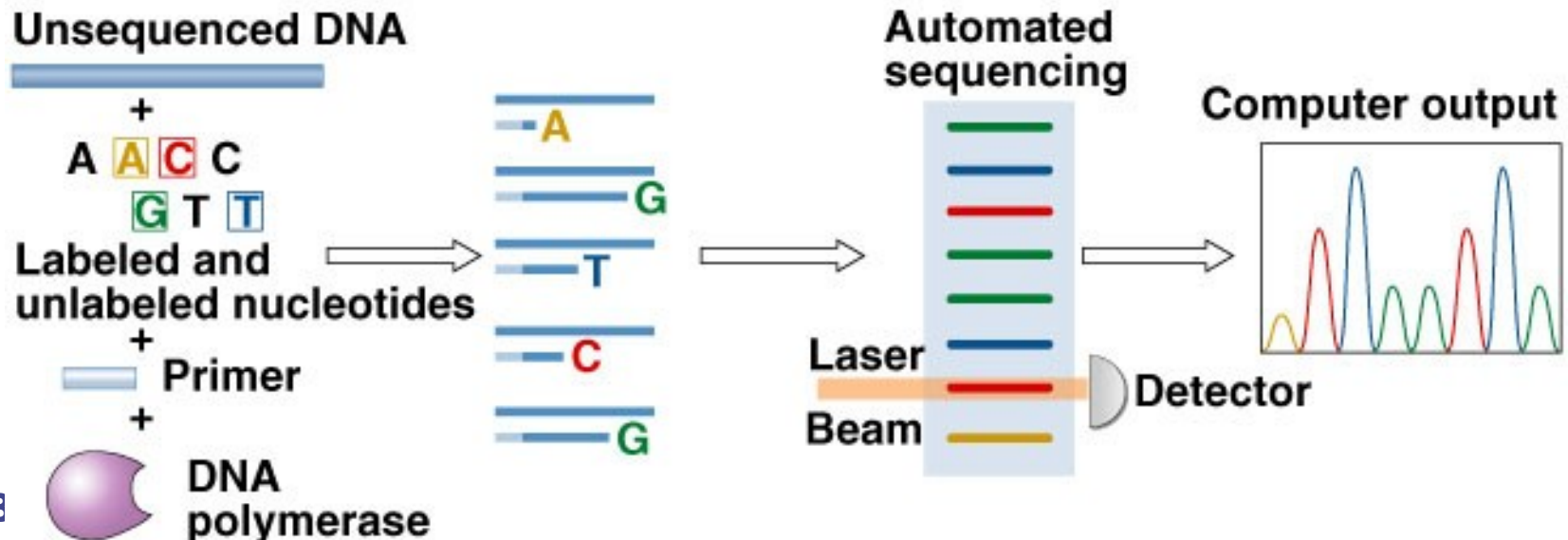
This was his 2nd Nobel Prize!!

- ◆ 1st was in 1958 for the structure of insulin



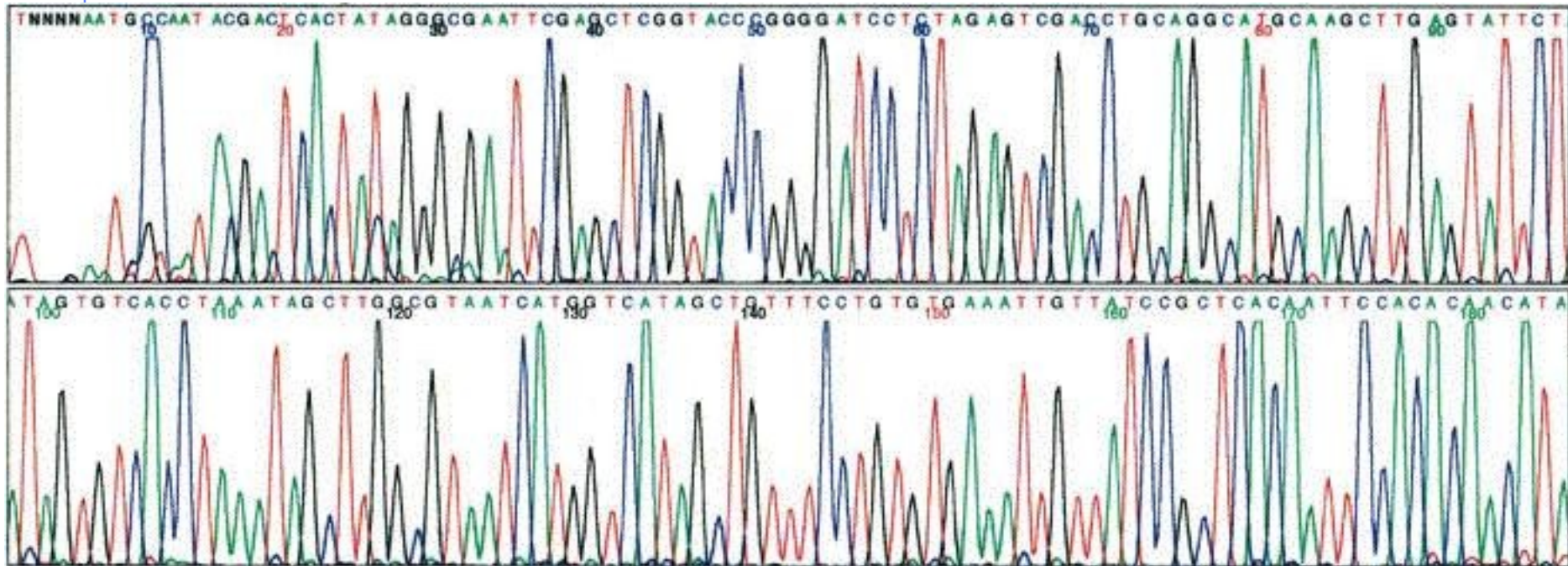
Advancements to sequencing

- **Fluorescent tagging**
 - ◆ no more radioactivity
 - ◆ all 4 bases in 1 lane
 - each base a different color
- **Automated reading**



Advancements to sequencing

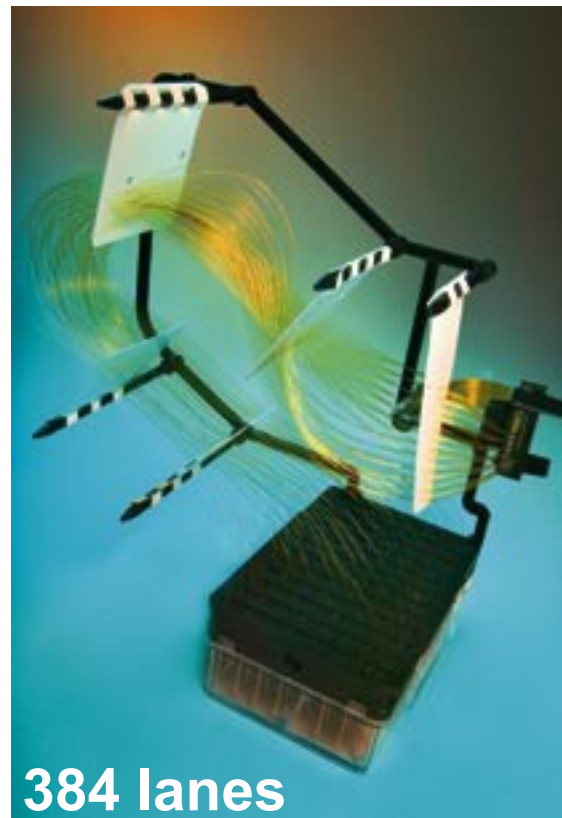
- Fluorescent tagging sequence data
- Computer read & analyzed



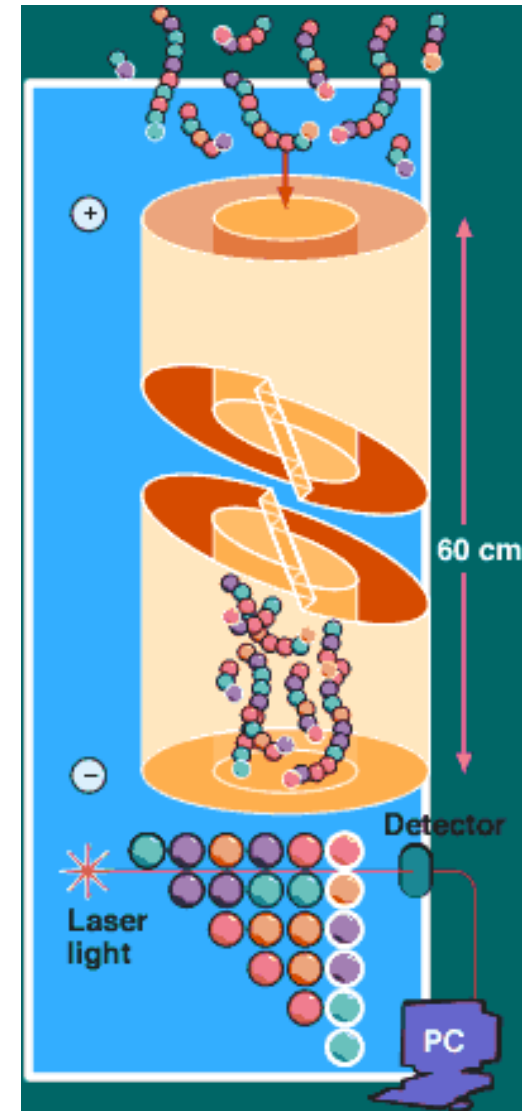
Advancements to sequencing

- Capillary tube electrophoresis
 - ◆ no more pouring gels
 - ◆ higher capacity & faster

Applied Biosystems, Inc (ABI) built an industry on these machines



384 lanes



■ Big labs!

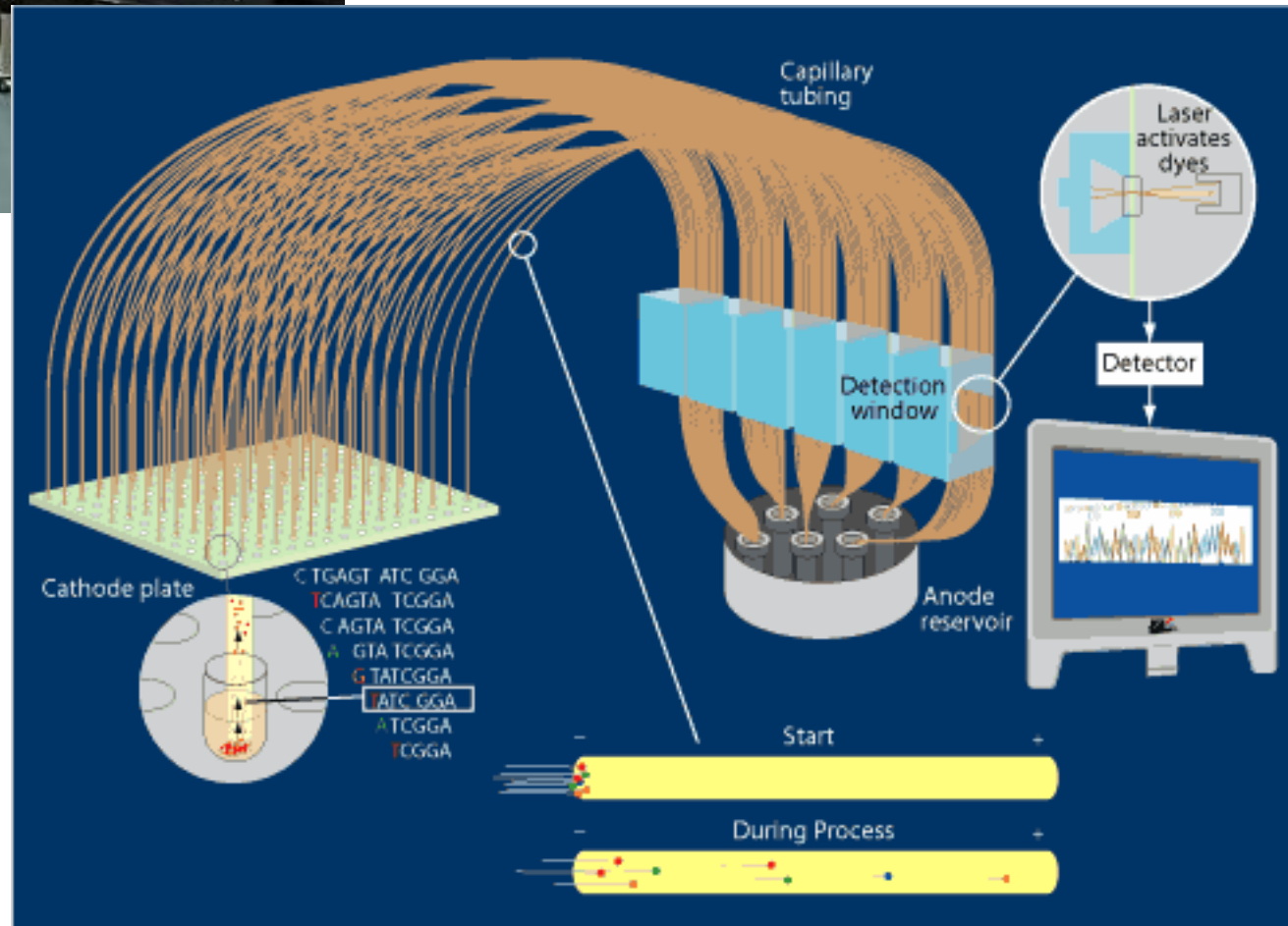
◆ economy of scale

PUBLIC

- Joint Genome Institute (DOE)
- MIT
- Washington University of St. Louis
- Baylor College of Medicine
- Sanger Center (UK)

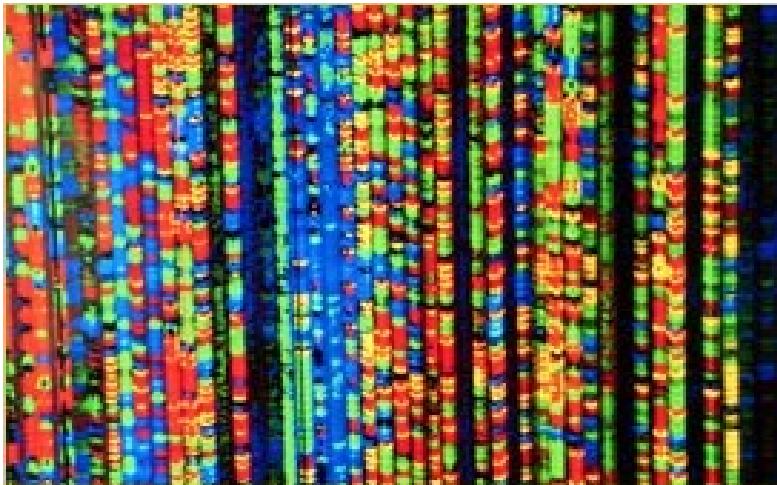
PRIVATE

- Celera Genomics



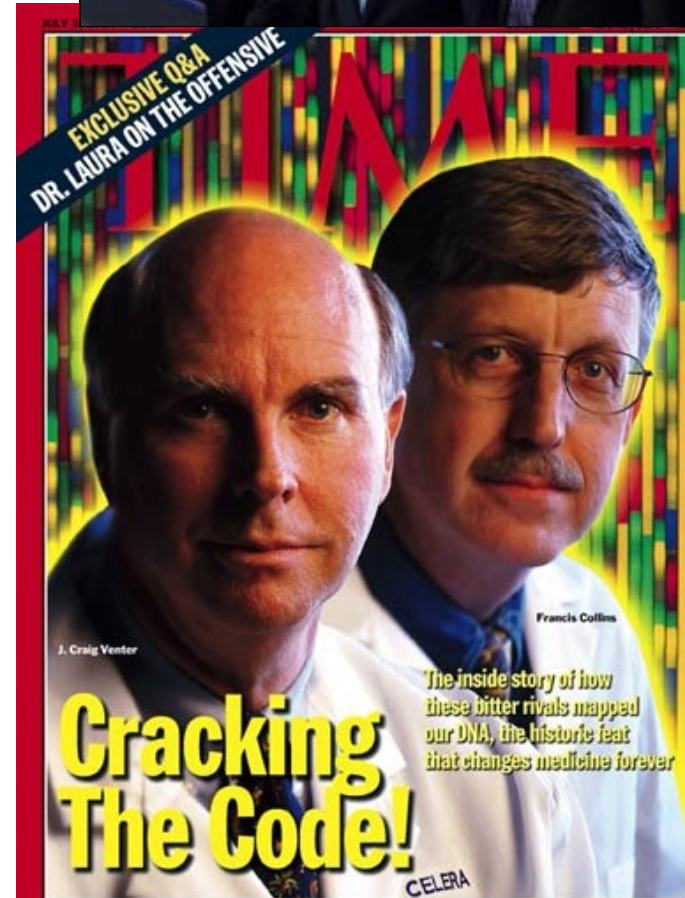
Automated Sequencing machines

- Really BIG labs!



Human Genome Project

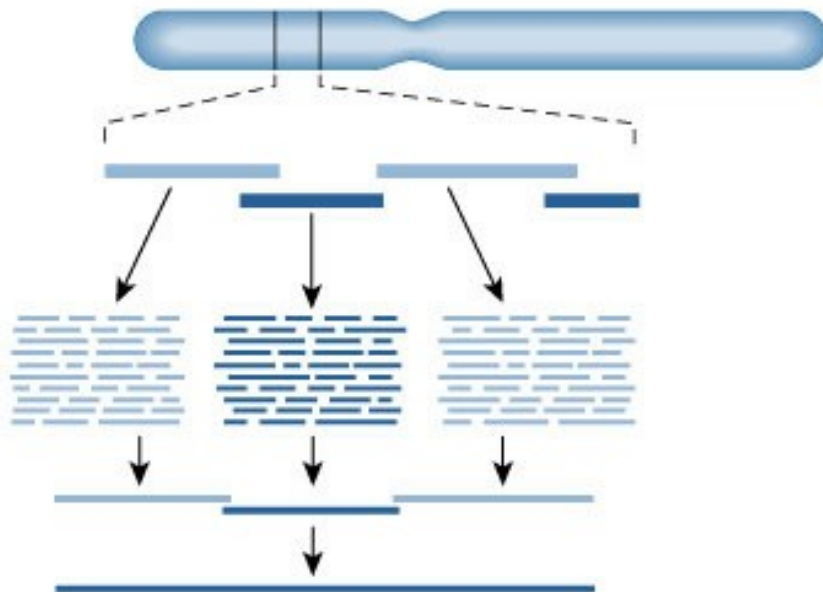
- U.S government project
 - ◆ begun in 1990
 - estimated to be a 15 year project
 - ◆ DOE & NIH
 - initiated by Jim Watson
 - led by Francis Collins
 - ◆ goal was to sequence entire human genome
 - 3 billion base pairs
- Celera Genomics
 - ◆ Craig Venter challenged gov't
 - ◆ would do it faster, cheaper
 - ◆ private company



Different approaches

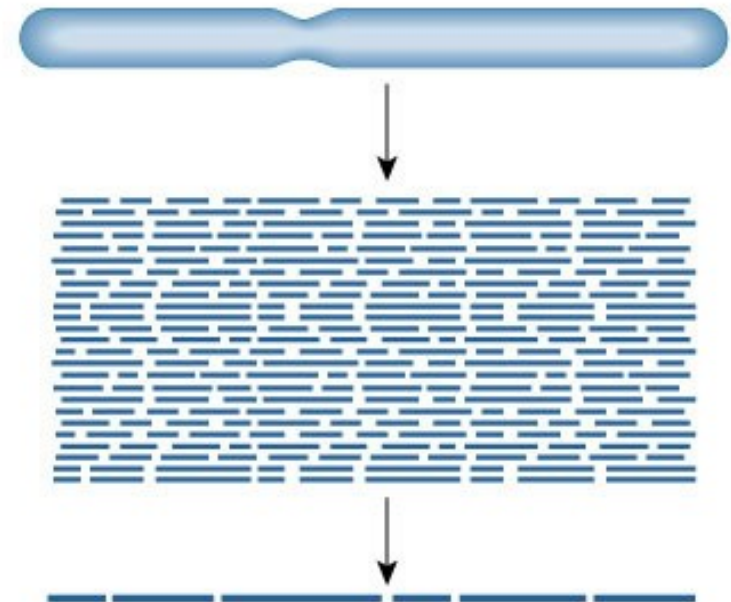
gov't method “map-based method”

1. Cut DNA segment into fragments, arrange based on overlapping nucleotide sequences, and clone fragments.
2. Cut and clone into smaller fragments.



Craig Venter's method “shotgun method”

1. Cut DNA entire chromosome into small fragments and clone.
2. Sequence each segment & arrange based on overlapping nucleotide sequences.

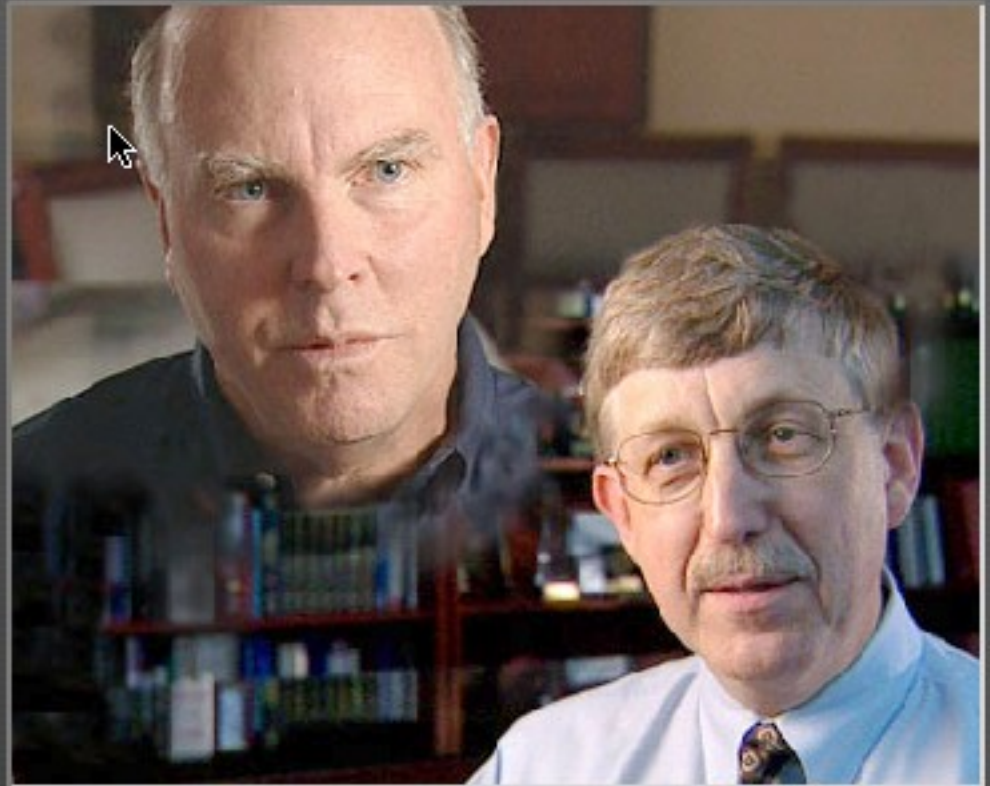


Human Genome Project

On June 26, 2001, HGP published the “working draft” of the DNA sequence of the human genome.

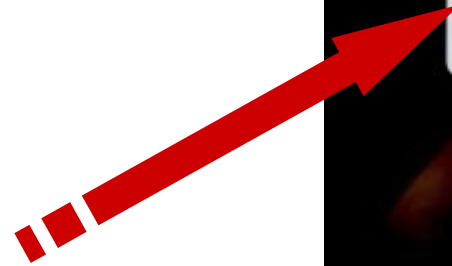
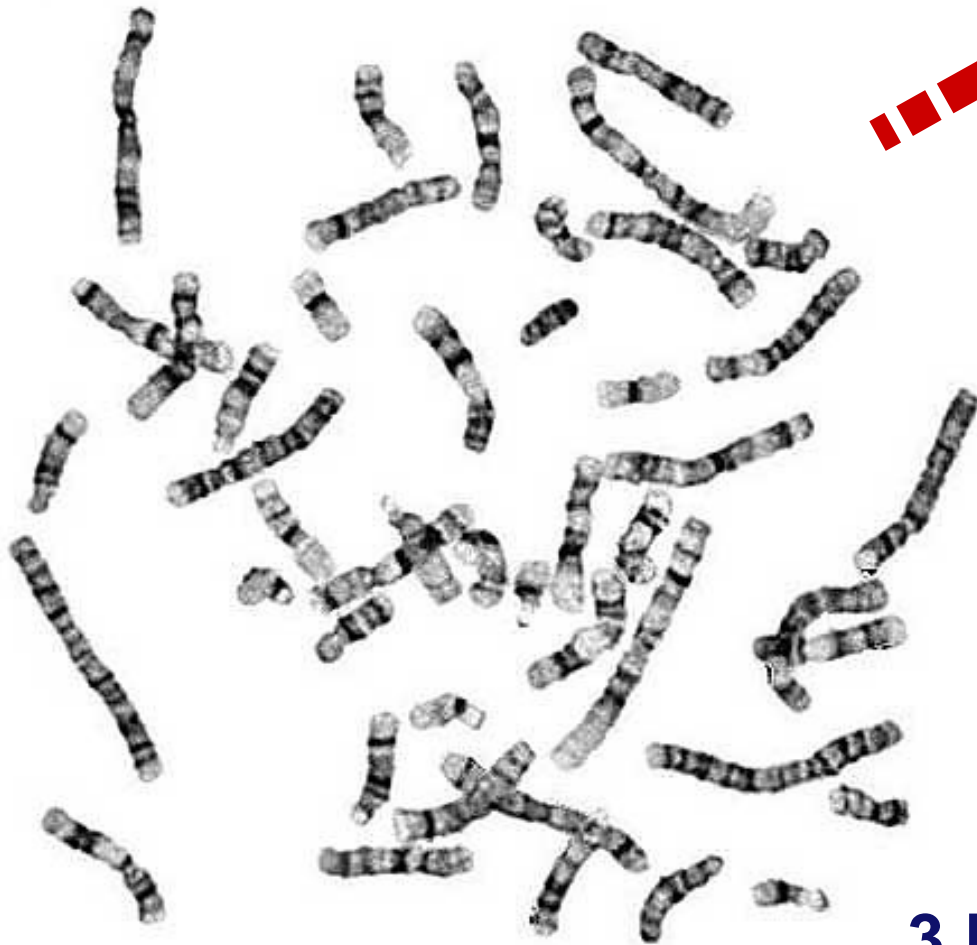
Historic Event!

- ◆ blueprint of a human
- ◆ the potential to change science & medicine



The leaders of the private and public genome projects, Craig Venter and Francis Collins.

Sequence of 46 Human Chromosomes



3G of data

3 billion base pairs

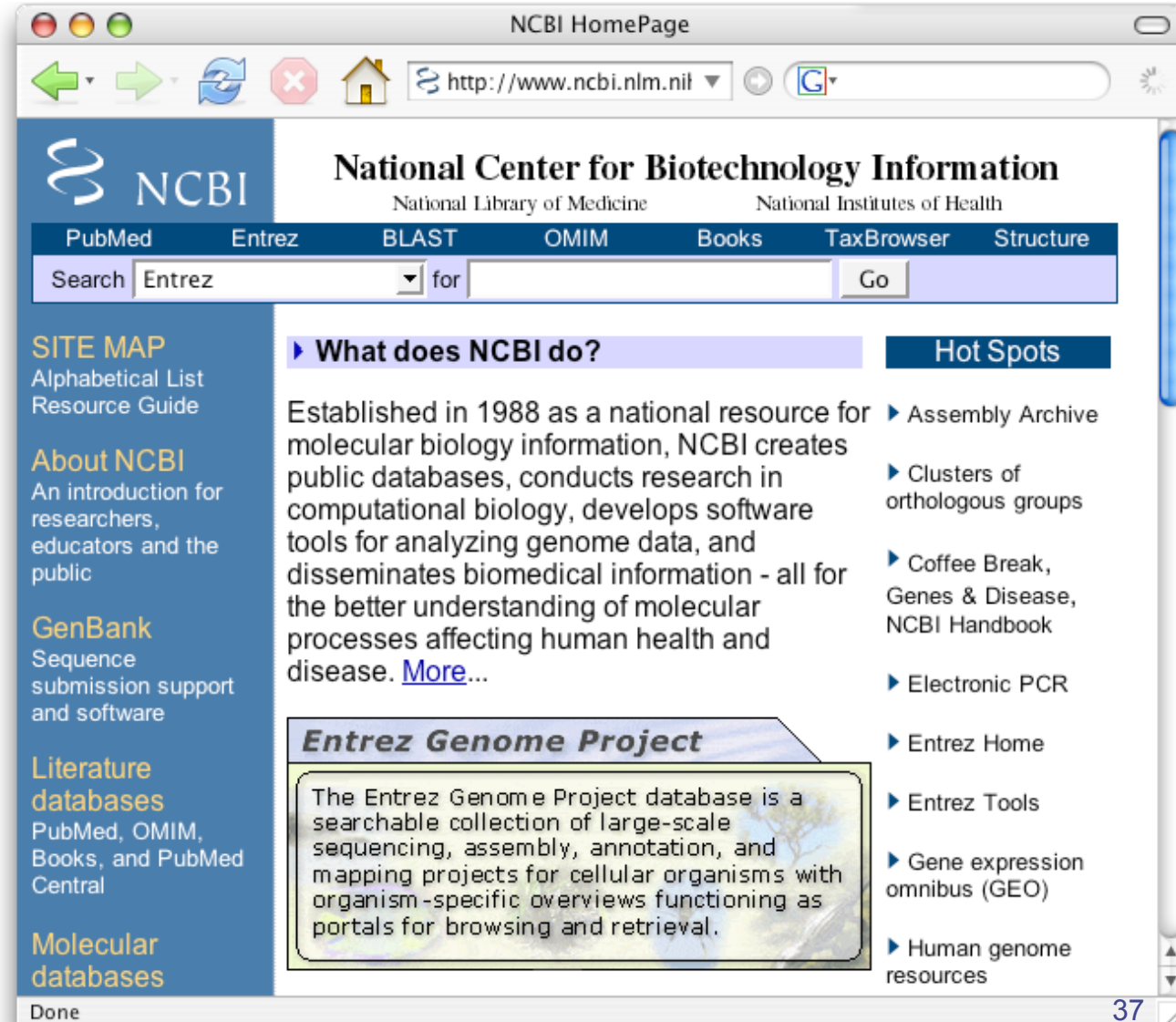
Raw genome data



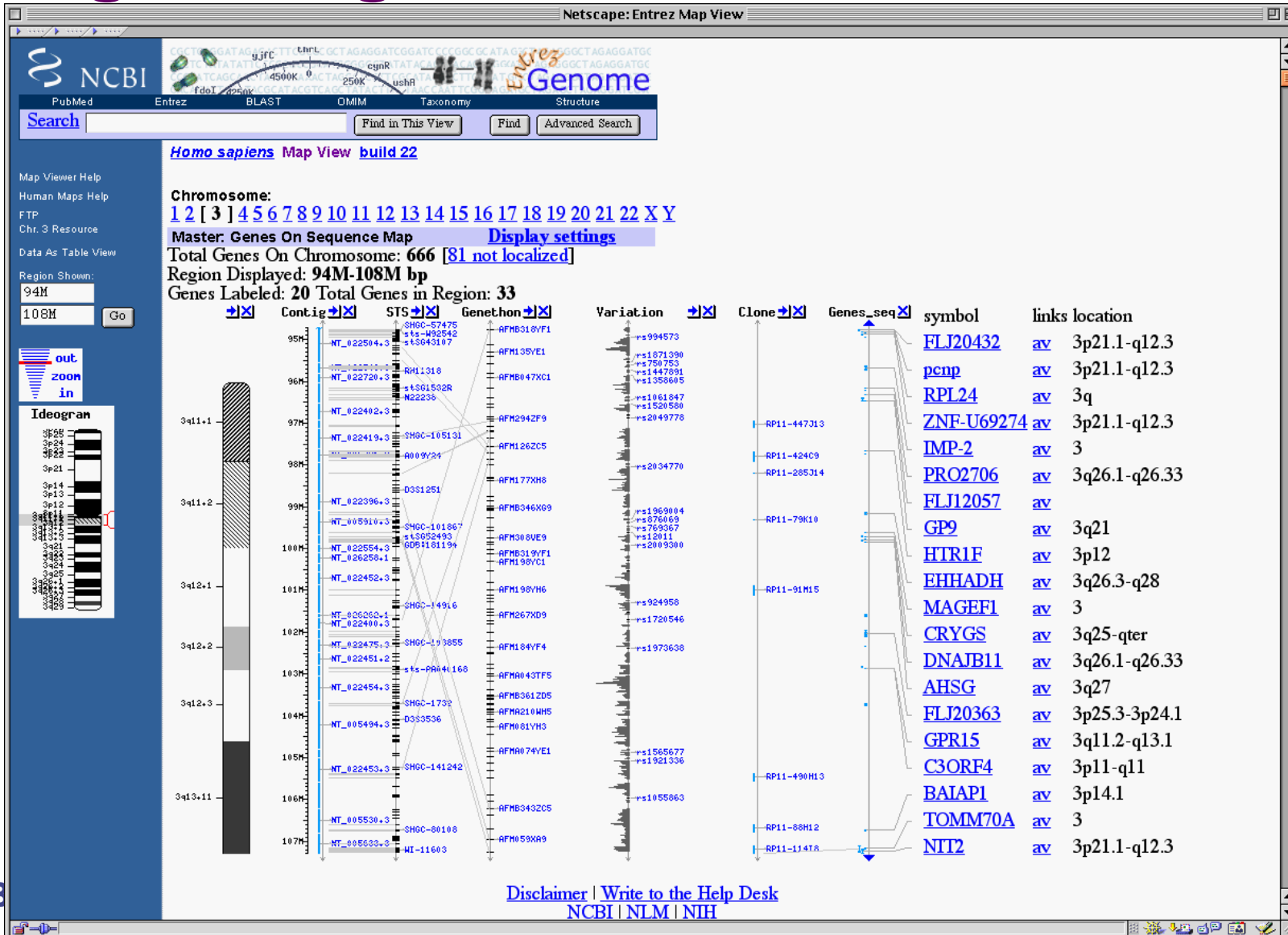
GenBank

Database of
genetic
sequences
gathered
from
research

Publicly
available!



Organizing the data



Maps of human genes...

- Where the genes are...
 - ◆ mapping genes & their mutant alleles

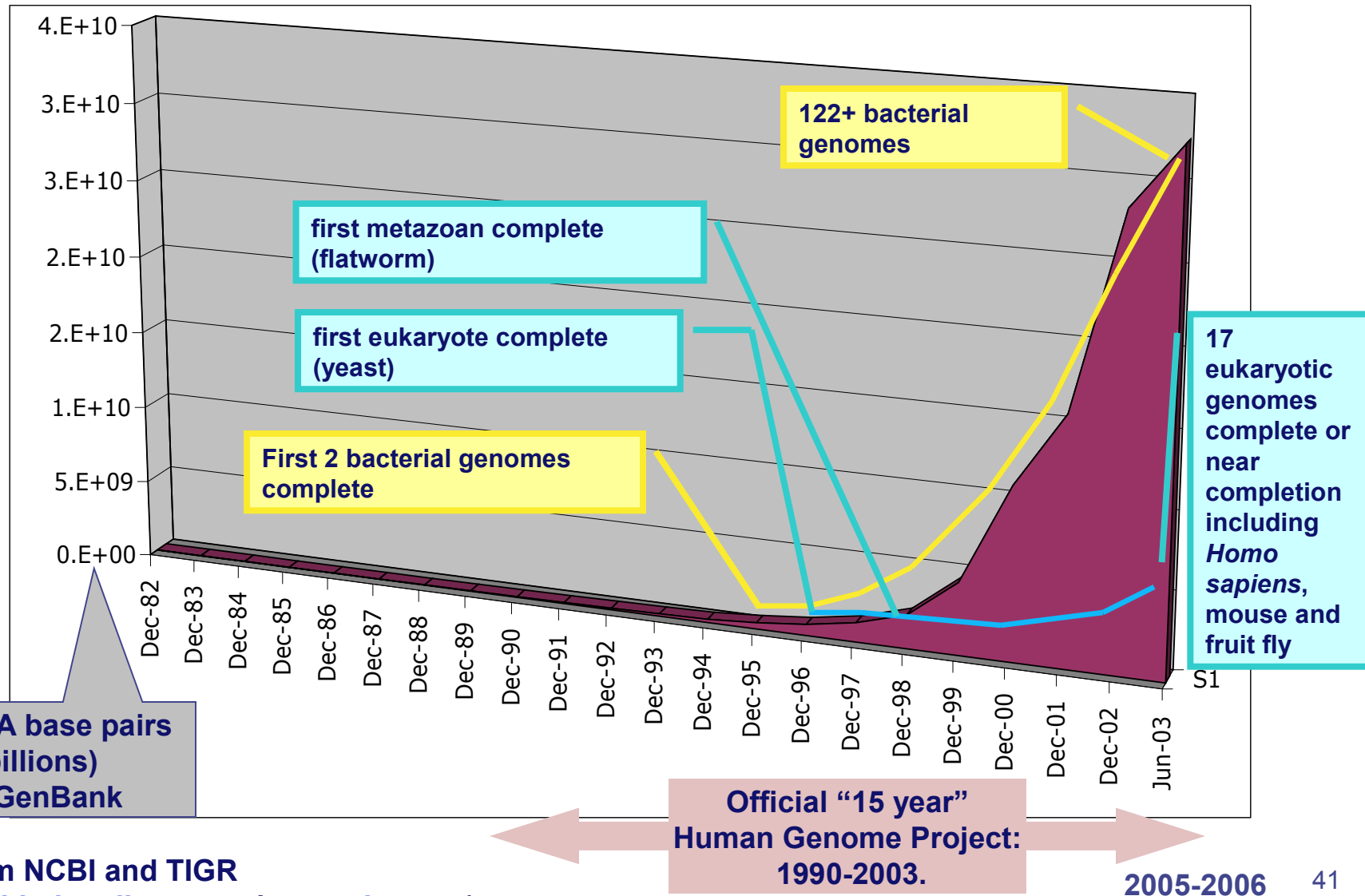
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



And we didn't stop there...



The Progress



How does the human genome stack up?

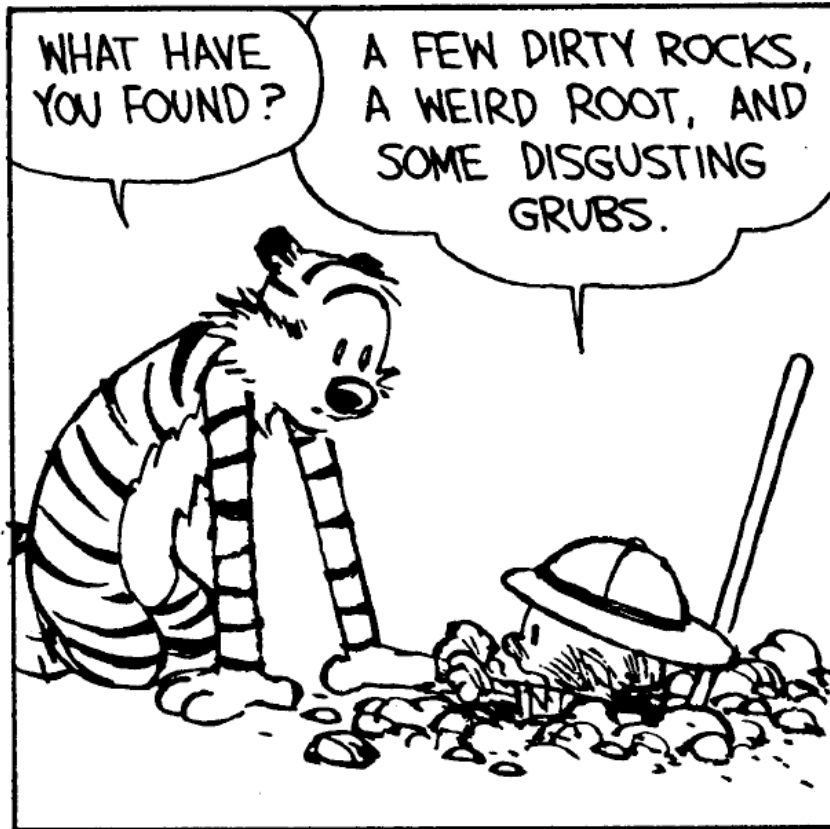
Organism	Genome Size (bases)	Estimated Genes
Human (<i>Homo sapiens</i>)	3 billion	30,000
Laboratory mouse (<i>M. musculus</i>)	2.6 billion	30,000
Mustard weed (<i>A. thaliana</i>)	100 million	25,000
Roundworm (<i>C. elegans</i>)	97 million	19,000
Fruit fly (<i>D. melanogaster</i>)	137 million	13,000
Yeast (<i>S. cerevisiae</i>)	12.1 million	6,000
Bacterium (<i>E. coli</i>)	4.6 million	3,200
Human Immunodeficiency Virus (HIV)	9700	9

What have we found?

- When you go looking...



...you will certainly find something!

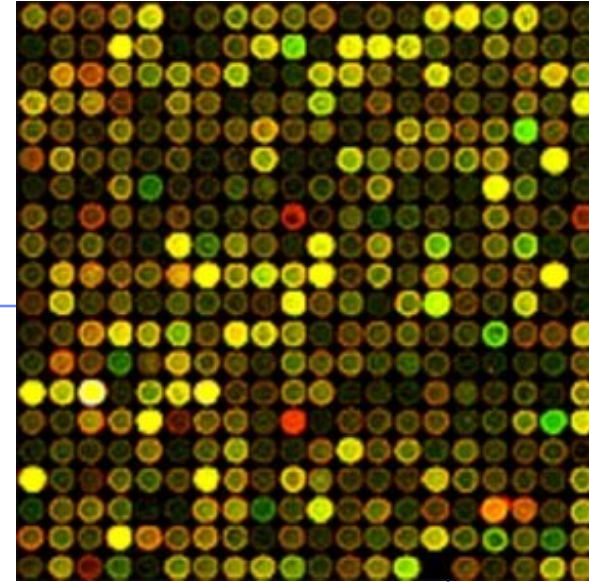




Any Questions??

Advanced Techniques

Microarrays

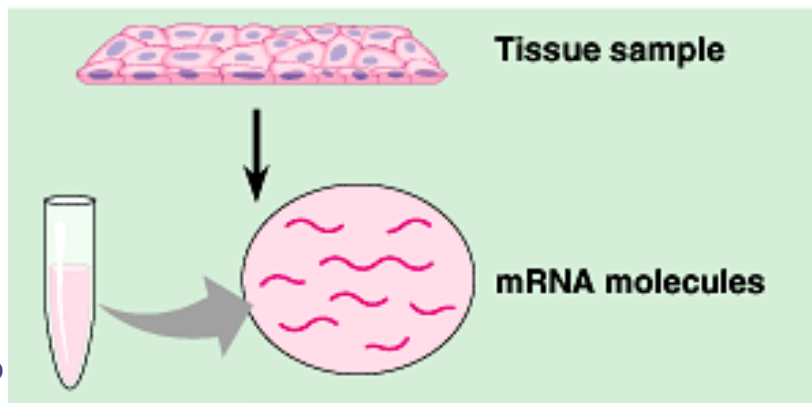


Where do we go next....

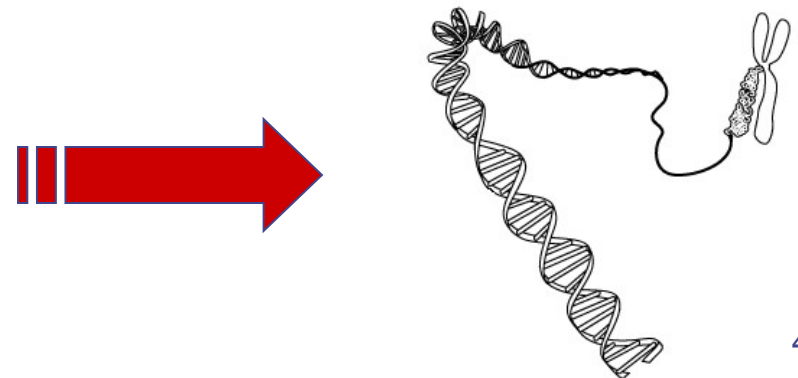
DNA → **RNA** → **protein** → **trait**

- When a gene is turned on, it creates a trait
 - ◆ want to know what gene is being expressed

extract mRNA from cells
mRNA = active genes

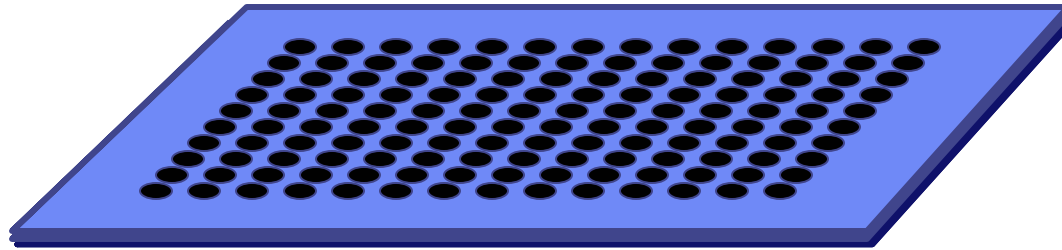


How do you match mRNA
back to DNA in cells???



Microarrays

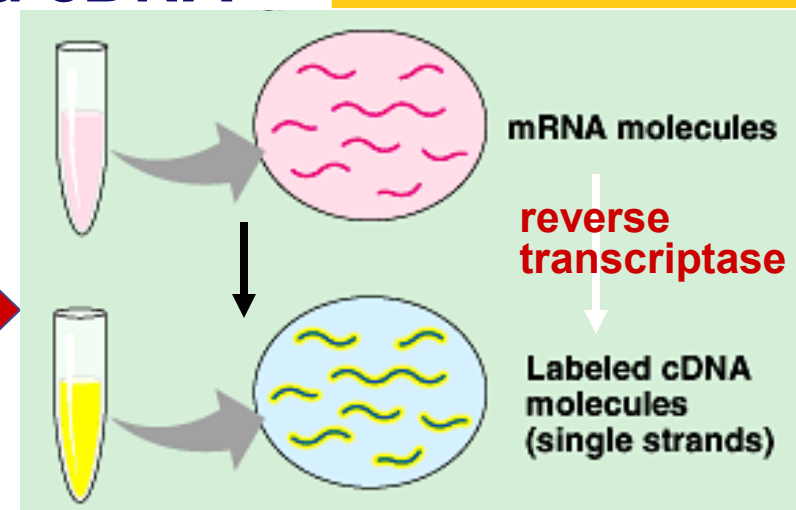
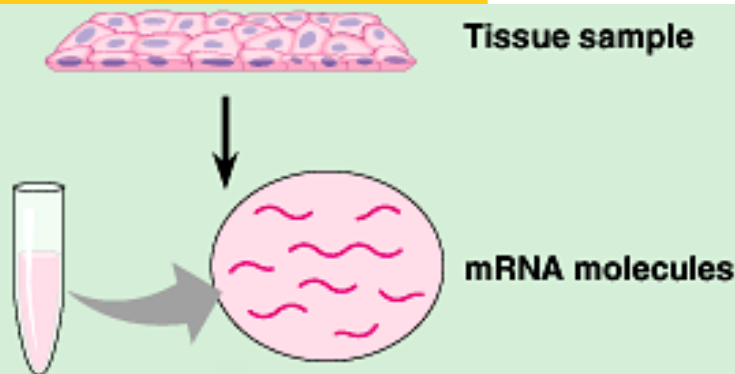
slide with spots of DNA
each spot = 1 gene



- Create a slide with a sample of each gene from the organism
 - ◆ each spot is one gene
- Convert mRNA → labeled cDNA

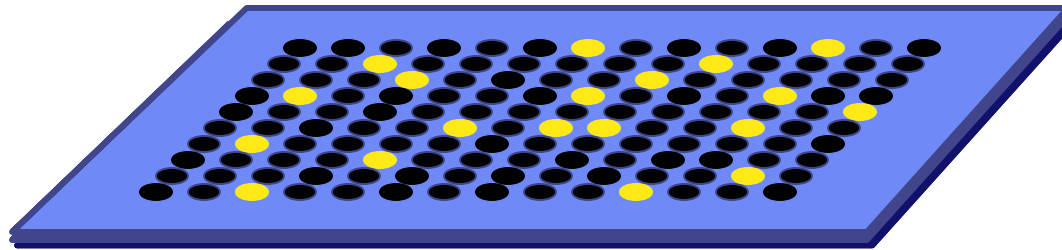
mRNA → cDNA

mRNA from cells



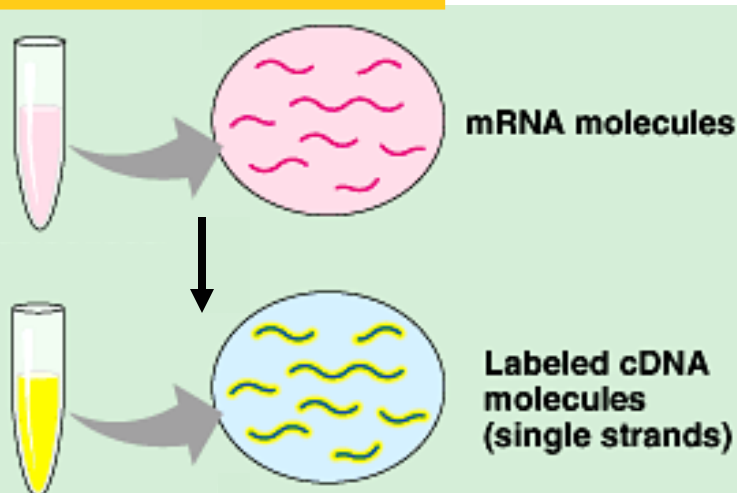
Microarrays

slide with spots of DNA
each spot = 1 gene

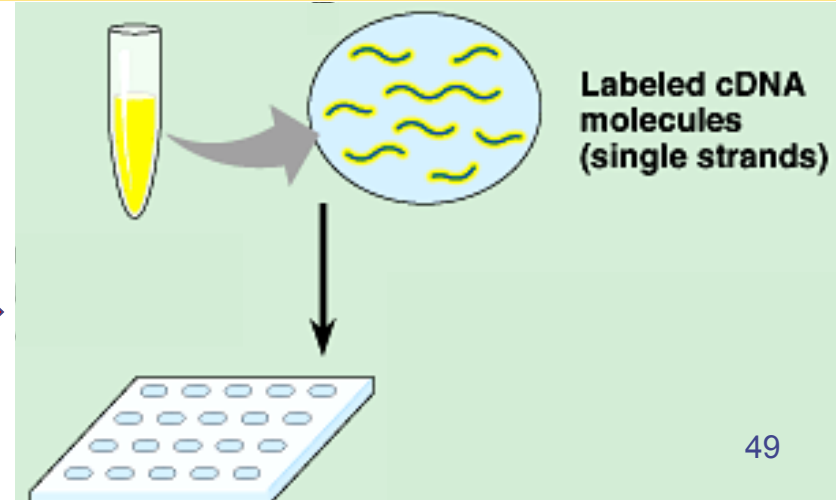


- Labeled cDNA hybridizes with DNA on slide
 - ◆ each **yellow** spot = gene matched to mRNA
 - ◆ each **yellow** spot = expressed gene

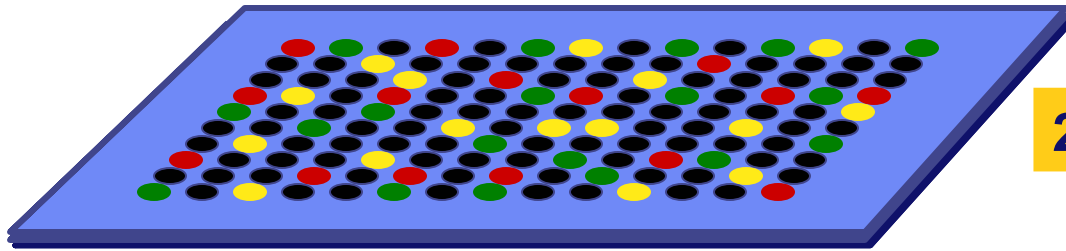
mRNA → cDNA



cDNA matched to genomic DNA



Application of Microarrays

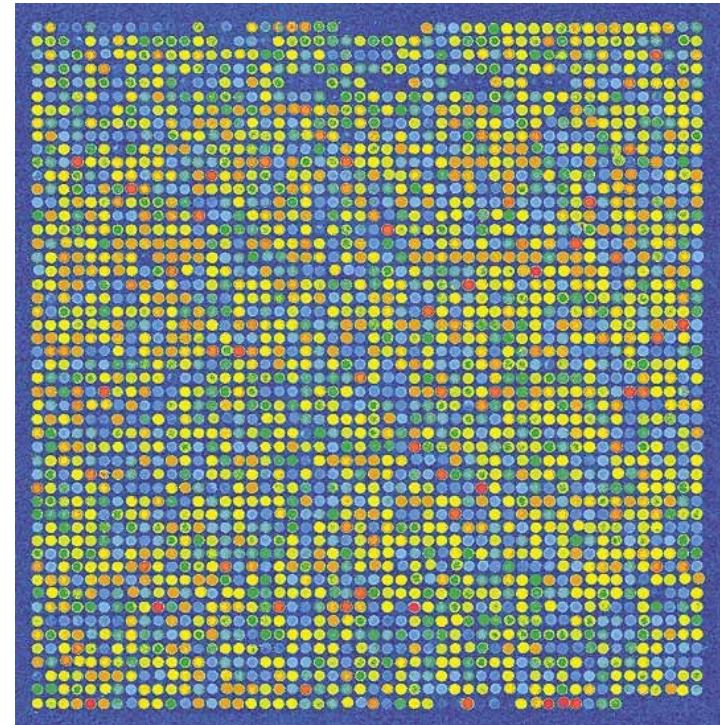


2-color fluorescent tagging

- Comparing treatments or conditions = Measuring change in gene expression
 - ◆ sick vs. healthy; cancer vs. normal cells
 - ◆ before vs. after treatment with drug
 - ◆ different stages in development
- Color coding: label each condition with different color
 - ◆ red = gene expression in one sample
 - ◆ green = gene expression in other sample
 - ◆ yellow = gene expression in both samples
 - ◆ black = no or low expression in both

“DNA Chip”

- **Patented microarray technology from Affymetrix**
 - ◆ **automated DNA synthesis of genes of interest on chip**
 - chips are more consistent
 - smaller spots/more spots per chip
 - ◆ **can buy specific chips**
 - human chip
 - mouse chip
 - etc.



Biotechnology today: Applications

- Application of DNA technologies
 - ◆ basic biological research
 - ◆ medical diagnostics
 - ◆ medical treatment (gene therapy)
 - ◆ pharmaceutical production
 - ◆ forensics
 - ◆ environmental cleanup
 - ◆ agricultural applications

...and then there's the ethics issues!

Application of recombinant DNA

- Combining sequences of DNA from 2 different sources into 1 DNA molecule
 - ◆ often from different species
 - human insulin gene in *E. coli* (humulin)
 - frost resistant gene from Arctic fish in strawberries
 - “Roundup-ready” bacterial gene in soybeans
 - BT bacterial gene in corn
 - jellyfish glow gene in Zebra “Glofish”



Human Cloning

Human cloning is very controversial & not the main goal of biotechnology





Any Questions??

What next?

- **After you have cloned & amplified DNA (genes), you can then tackle more interesting questions**
 - ◆ **how does gene differ from person to person?**
 - ...or species to species
 - ◆ **is a certain allele associated with a hereditary disorder**
 - ◆ **in which cells is gene expressed?**
 - ◆ **where is gene in genome?**